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18th Congress of the European
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PLENARY LECTURES

> PL1. Plenary Lecture 1

Presidential Lecture

MICROBIAL RHODOPSINS: DIVERSITY, MECHANISMS, AND OPTOGENETIC APPLICATIONS

Author: John Spudich[1]
1) Center for Membrane Biology, Department of Biochemistry and Molecular Biology, University of Texas Health Science Center. McGovern Medical School, Houston, TX, USA

Microbial rhodopsins are a family of photoactive retinylidene proteins widespread throughout the microbial world. They are notable for their diversity of function, using variations of a shared seven-transmembrane helix design and similar photochemical reactions to carry out distinctly different light-driven energy and sensory transduction processes. Their study continues to advance our understanding of how evolution modifies protein scaffolds to create new protein chemistry. An emerging principle, elegant in its simplicity, is becoming evident from comparative analysis of their atomic structures and interconversion of their functions by site-specific mutagenesis, namely, that subtle modifications of the atomic structure of their photoactive sites are central to producing large differences in their molecular functions.

Use of microbial rhodopsins as genetically targeted tools to control membrane potential with light has given rise to the new technology of optogenetics, which has had transformative impact on research in neurophysiology. Cation-conducting channelrhodopsins (CCRs) enabling targeted photoinduced neuron firing and light-driven transporters and more recently anion-conducting channelrhodopsins (ACRs) enabling neuron photosuppression have become established as effective tools for analysis of brain circuitry. Microbial rhodopsins have also begun to be tested for optogenetic gene therapy in animal models of neurological diseases. A promising clinical effort in progress is microbial opsin-based vision restoration to the blind. Human clinical trials using targeted expression of channelrhodopsins in the retina of individuals blind from the retinal degenerative disease retinitis pigmentosa have begun.

Progress on ACRs will be presented that impacts our understanding of light-gated channel conductance as well as neural inhibition applications optogenetics. Our crystal structure of *Gt*ACR1 from *Guillardia theta* revealed a continuous intramolecular tunnel traversing the protein from its extracellular to its cytoplasmic surface, predicted to expand to form the anion-conducting channel upon photoactivation. This finding has led to new insights into the conductance mechanism.

References

Li H, Huang CY, Govorunova EG, Schafer CT, Sineshchekov OA, Wang M, Zheng L, and Spudich JL (2019) Crystal structure of a natural light-gated anion channelrhodopsin. *Elife* 8:e41741.

Govorunova EG, Sineshchekov OA, Li H, and Spudich JL (2017) Microbial Rhodopsins: Diversity, Mechanisms, and Optogenetic Applications. *Annu. Rev. Biochem.* 86:845-872.

Govorunova EG, Sineshchekov OA, Janz R, Liu X, and Spudich JL (2015) Natural light-gated anion channels: A family of microbial rhodopsins for advanced optogenetics. *Science* 349:647-650.

> PL2. Plenary Lecture 2

Young Investigator Award

THE ENTHRALLING CHALLENGE OF HARVESTING PHOTOEXCITED ELECTRONS FROM INTACT BACTERIAL CELLS

Author: Matteo Grattieri[1]
1) University of Utah

Coupling of photosynthetic biological entities with electrode surfaces for sun energy conversion into electrical energy has attracted a tremendous interest in the scientific community.[1-4] Particularly, the application of intact photosynthetic bacterial cells provides all the necessary enzymes and cofactors for photosynthesis, while enhancing stability and functionality thanks to the self-repair mechanisms of microorganisms.[5] The challenge for their application is the extraction and transfer of the photoexcited electrons to an electrode, a process defined “extracellular electron transfer”, for which little information is available for the great majority of bacterial species.[6]

In this context, the possibility to establish an electrochemical communication between purple bacteria and an electrode is of extreme interest, as their versatile metabolism could enable sun powered bioelectrochemical systems for energy production while performing the removal and monitoring of a broad variety of contaminants. During the lecture pioneering studies of purple bacteria bio-photoelectrocatalysis will be introduced, to then discuss the extracellular electron transfer process of these microorganisms. Following this introduction, artificial approaches to enhance bio-photocurrent generation will be presented, unveiling how bio-inspired biotic/abiotic photo-anode can be utilized to enhance photo-bioelectrocatalysis while developing suitable systems for application in the field. Finally, future challenges and research needs for the community of bio-photoelectrochemical systems will be discussed.

References

1. A. J. McCormick, P. Bombelli, R. W. Bradley, R. Thorne, T. Wenzel and C. J. Howe, *Energy Environ. Sci.*, **8**, 1092 (2015).
2. G. Longatte, A. Sayegh, J. Delacotte, F. Rappaport, F. A. Wollman, M. Guille-Collignon and F. Lemaitre, *Chem. Sci.*, **9**, 8271 (2018).
3. D. Ciornii, M. Riedel, K. R. Stieger, S. C. Feifel, M. Hejazi, H. Lokstein, A. Zouni and F. Lisdat, *J. Am. Chem. Soc.*, **139**, 16478 (2017).
4. F. Milano, A. Punzi, R. Ragni, M. Trotta and G. M. Farinola, *Adv. Funct. Mater.*, 1805521 (2018).
5. S. Malik, E. Drott, P. Grisdela, J. Lee, C. Lee, D. A. Lowy, S. Gray and L. M. Tender, *Energy Environ. Sci.*, **2**, 292 (2009).
6. G. Pankratova and L. Gorton, *Curr. Opin. Electrochem.*, **5**, 193 (2017).

> PL3. Plenary Lecture 3

Edna Roe Lecture

LOW LEVEL SOLAR UVR EFFECTS ON HUMAN SKIN ACROSS PHOTOTYPES I-VI: BENEFIT AND HARM

Author: Lesley Rhodes[1]
1) University of Manchester and Salford Royal Hospital

Sunlight has many established and emerging effects on human skin, their mediation including by cyclobutane dimer (CPD) DNA damage and immune and endocrine activities.

The best-established benefit is initiation of vitamin D synthesis, and the greatest harm to skin is malignancy, with sunburn erythema adopted as proxy for DNA damage/skin cancer. Several questions were unanswered including: relationship between sun exposure and vitamin D status; value of sunburn as proxy; influence of skin type/colour.

Sunburn is an inflammatory response triggered by DNA damage. Even one episode can have prolonged effect on skin homeostasis. It's widely used in sun exposure advice, i.e. to keep below personal visual erythema threshold (MED). However, assessment by laser speckle contrast imaging of blood flux revealed a higher level of sensitivity in dark skin, and a robust method was defined for determining sunburn threshold through skin type I-VI.

A series of statistically justified in vivo studies (total 1150 subjects) examined UVR-vitamin D relationship, to both absolute and MED-related UVR dose. Interventions were under simulated summer conditions and linked to natural exposure (53.5°N). First, multiple UVR doses (1.3SED), equivalent to 10-15 minutes at midday, were shown to provide vitamin D sufficiency in light-skin people, if acquired regularly to ~35% skin; face and hands exposure was inadequate.

Next, the question whether melanisation reduces vitamin D synthesis was addressed. Yes, under realistic conditions, when repeatedly given the same absolute low UVR doses, rise in 25(OH)D was lower in dark- vs light-skin people ($p<0.0001$). In contrast, when dosed according to individual's MED, the same 25(OH)D gain was seen. Indeed, dose-response studies showed attainable levels of solar UVR (1.95 SED) corrected vitamin D deficiency in dark-skin people.

Then, the amount of epidermal DNA damage occurring with repeated 1.3SED exposures was explored; DNA damage ensued but with repair, i.e. no CPD accumulation over a 6-week course in light or dark skin types, providing some reassurance.

More complexity was revealed in a UVR dose-response study (0.2-0.8MED) in skin types I-VI. Same total level of epidermal DNA damage, erythema and 25(OH)D occurred regardless of skin type. However, a striking gradient of epidermal CPD was discovered that correlated with skin colour ($r=0.74$, $p<0.0001$). Thus 25(OH)D gain: basal cell damage balance was increasingly favourable towards darker skin types. In lighter skin, basal damage occurred with vitamin D synthesis even at exquisitely low UVR level (0.2MED; i.e. only 0.5 SED in type I/II), suggesting extra caution for the lightest skin types.

Emerging data supports low level UVR-induction of other mediators e.g. endocannabinoids. While the Montreal Protocol has limited the increases in ambient UVB caused by stratospheric ozone depletion, beneficial and harmful effects on skin appear with very low UVR levels.

> PL4. Plenary Lecture 4

Finsen Lecture

FROM FINSEN TO PhoCIS: HOW SUNLIGHT INFLUENCES IMMUNE-MEDIATED DISEASES

Author: Scott Byrne[1,2]
1) The University of Sydney, Faculty of Medicine and Health, Sydney, Australia 2) The Westmead Institute for Medical Research, Sydney, Australia

The ultraviolet (UV) radiation contained in sunlight is well-known for causing skin cancer. What is less appreciated is that increasing one's exposure to UV has a number of important health benefits including protecting us from depression, autoimmunity and cardiovascular disease. UV is a powerful and broad-spectrum immune suppressant which likely explains its ability to exert both detrimental and beneficial effects.

Our team studies the mechanisms underlying UV-immune suppression as this will ultimately allow us to block, replicate and/or enhance the UV-effect. To that end we have discovered that molecular signals generated in UV-exposed skin leads to the activation of a unique subset of regulatory B cells in lymphoid organs. These cells, which we call "UV-B-Regs" were major cellular players in UV-carcinogenesis, as depleting them with an antibody led to improved survival and less metastases in skin tumour-bearing mice. In a different disease context, UV-B-Regs were found to be responsible for UV-protection from an autoimmune attack on the central nervous system of mice. Thus, UV-B-Regs are a key indirect target of UV radiation and targeting these cells is a novel way to influence immune-mediated diseases.

Balancing the human need for sunlight against the recognised harmful effects is a major health challenge. Understanding how UV modulates our immune system is important if we are to harness the power of sunlight to prevent and treat chronic disease safely, without risking an increase in skin cancers.

> PL5. Plenary Lecture 5

Finsen Medal Award Lecture

ENVIRONMENTAL PHOTOBIOLOGY: UV RADIATION, CLIMATE RISKS AND CHALLENGES FOR SUSTAINABILITY

Author: Janet F. Bornman[1]
1) Murdoch University

Photobiology of animal and plant response to solar radiation has become a multi-disciplinary science, increasing in complexity through its progression from studies of single cells and individual organisms to terrestrial and aquatic ecosystems. This emergence of environmental photobiology is a key indicator of current and potential consequences of a rapidly changing environment that is posing both threats and advantages to life on Earth, as well as to non-living systems such as societal infrastructure.

A common challenge for most life on Earth is finding a balance between too much and too little solar radiation, with UV radiation playing a decisive role. For example, beneficial effects of the UV-induction of vitamin D, have been linked to reduced risk of musculoskeletal disorders, some internal cancers, innate autoimmune diseases, and non-cancerous skin diseases(1). Also, the UV-stimulated production in plants of secondary metabolites, such as phenolics, act both as plant screening compounds, antioxidants, and pest deterrents(2), as well as being nutritionally useful for animals including humans(3,4). On the other hand, UV radiation and the modifying effects of changing climate are increasing incidences of skin cancer and eye diseases in humans(1). Many aquatic organisms are vulnerable to high levels of UV radiation and interactive effects of environmental change, resulting in significant loss of productivity(5). Effects on terrestrial ecosystems, including agriculture, appear to be less damaging, although they can substantially alter ecosystem functioning(3).

The evolving complexity of environmental photobiology is heightened by the interactive effects of rapid climate change and those of stratospheric ozone dynamics, which in turn affect UV radiation received at the Earth's surface. These events result in positive and negative feedbacks between UV radiation and climate, with consequences for life on Earth.

This presentation will journey through some of the challenges for maintaining a sustainable future against a background of increasing complexity and interactions with respect to the response to UV radiation and environmental change. Are we on track towards a sustainable environment in line with the UN Sustainable Development Goals?

References

1. Lucas, R.M., Yazar, S., Young, A.R., et al. 2019. Photochem. Photobiol. Sci., 18, 641-680.
2. Escobar-Bravo, R., Klinkhamer, P.G. & Leiss, K.A. 2017. Frontiers Plant Sci., 8, 278.
3. Bornman, J.F., Barnes, P.W., Robson, T.M., et al. 2019. Photochem. Photobiol. Sci., 18, 681-716.
4. Wu, G., Bornman, J.F., Bennett, S.J., et al. 2017. J. Cereal Sci., 77, 17-23.
5. Williamson, C.E., Neale, P.J., Hylander, S., et al.2019. *Photochem. Photobiol. Sci.*18, 717-746.

> PL6. Plenary Lecture 6

USING LIGHT DEEP IN THE BODY

Author: S.H. Andy Yun[1]
1) Harvard Medical School / Wellman Center for Photomedicine, MGH

To fully realize the power of photobiology for medical applications, light must be delivered to target tissues with sufficient energy and specificity. This requirement poses technical challenges. In this talk, we will overview various emerging strategies for delivering optical energy into the body. Beyond conventional fiber-optic catheters and endoscopes, the efforts include development of biomaterial-based waveguides that are flexible, biocompatible, and even biodegradable. Light-controlled therapy and sensing have been demonstrated by using light-guiding hydrogel implants with fluorescent reporters and optogenetic cells. Multifunctional optical fibers may be implanted for neuromodulation of the nervous system in the body. Furthermore, unconventional light sources, such as bioluminescence and cellular lasers, may offer new ways of enabling and harnessing photobiology.

> PL7. Plenary Lecture 7

Finsen Medal Award Lecture

TIME-RESOLVED ENERGETICS AND STRUCTURAL VOLUME CHANGES IN PHOTOSENSOR PROTEINS

Author: Silvia Braslavsky[1]
1) Max Planck Institute for Chemical Energy Conversion, Germany

In retinal proteins, in photoactive biliproteins such as phytochromes and in other photonic signal transducers, the energy stored in the first thermodynamically stable intermediate after primary photochemistry, formed in the sub-ns time scale, drives the rest of the photocycle. Time-resolved photothermal methods, such as laser-induced optoacoustics (LIOAS) have the unique ability to monitor the energy level of transient species and the corresponding structural volume changes of non-thermal origin, such as water rearrangements induced by changes of dipolar moment after photoisomerization.[1],[2],[3] LIOAS was used to determine the energy content of nano- and µs species and the structural volume changes accompanying their formation and decay in retinal proteins, PYP, and in plant phytochrome A from Avena.[2] Lately, the 65 kDa truncated form (*As*phyA) assembled with phytochromobilin and with phycocyanobilin, as well as diverse photochromic bilin-binding photoreceptors of prokaryotic origin were analysed by LIOAS.[4] This latter photoreceptors show large spectral versatility, novel physiological functions, and are appropriate for optogenetics and nanoscopies. The chromophore-binding domain of a red/green switching cyanobacteriochrome from *Synechocystis* (*Slr*1393g3), the red/far red *Synechocystis* Cph1 phytochrome, as well as full-length and truncated constructs of *Xantomonas campestris* bacteriophytochrome show similar prompt heat dissipation (³70%) in the sub-ns time scale upon formation of the first intermediate, reflecting the low quantum yield of photoisomerization. Plant *As*phyA shows a quantum yield of 0.17,[5] in the three bilin-binding proteins measured lately it is £ 0.3. Upon production of the first intermediate an expansion is produced of ca. 5-12 ml/mol, underscoring the relevance of geometric and steric effects. An exception is the green-absorbing form of *Slr*1393g3, the prompt expansion is followed by a contraction, indicating more mobility of water molecules in its chromophore cavity.cavit

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References

[1] Losi, A., Braslavsky, S.E. *Phys. Chem. Chem. Phys.*, **5**, 2739 (2003).
[2] Braslavsky, S.E., Heibel, G.E. *Chem. Rev.* **92**, 1381(1992).
[3] Gensch, T.,Viappiani, C., Braslavsky, S.E. in *Encyclopedia of Spectrosc. and Spectrom.* (Jan Reedijk, Editor-in-Chief) Elsevier Ref. Module. Chem., Molec. Scs. and Eng., (2014)
[4] Losi, A., Bonomi, H.R, Michael, R, Tang, K., Zhao, K.-H. *Photochem. Photobiol.* **93**, 733 (2017).
[5] Gensch, T., Churio, S., Braslavsky, S.E., Schaffner, K. *Photochem. Photobiol.* **63**, 719 (1996).

> PL8. Plenary Lecture 8

Finsen Medal Award Lecture

THE FUTURE OF PHOTODERMATOLOGY

Author: Henry W. Lim[1]
1) Department of Dermatology, Henry Ford Hospital, Detroit, Michigan, USA

From the time that Finsen was awarded Nobel Prize in 1903 for his discovery of using “light radiation” for the treatment of diseases, the subspecialty of photodermatology and photomedicine has made significant advances. Some of the new developments will be covered in the lecture:

Photobiology in medicine:
The effects of ultraviolet on skin has been well studied, which include erythema, tanning, photoaging and photocarcinogenesis. In the past few years, visible light has been shown to have clinically relevant biologic effects on the skin, including erythema and tanning. The use of photons as a tool to study skin optics (diffuse reflectance spectroscopy, confocal microscopy, multiphoton photothermolysis) are now actively utilized and investigated.

Photoprotection:
Modern sunscreens have been in used for over 40 years. Recently, endocrinologic effect and environmental impact of UV filters have generated much coverage by the media. Study by US FDA showed absorption of UV filters under maximal use condition, and US FDA has requested additional safety data on 12 of the UV filters, including 7 widely used ones. These new reports need to be addressed in public education on sun-safe behavior. Emerging data on systemic photoprotection agents (afamelanotide, nicotinamide, *Polypodium leucotomos*) show the possible role of adjuvant agents in photoprotection.

Phototherapy:
Narrowband UVB, targeted phototherapy, UVA1, psoralen and UVA (PUVA) have long been used in dermatology for the treatment of dermatoses; they are an integral part of the therapeutic options in dermatology. The advancements of laser for medical and aesthetic indications, as well as means for percutaneous drug delivery, have benefited patients worldwide. However, there are indications that not all dermatology trainees are comfortable in using phototherapy. This is a topic that we need to correct as educators.

In summary, much advances have been made in photodermatology that benefited our patients. We do need to continue our efforts in training and engaging the next generation of photodermatologists.

KEYNOTE LECTURES

> KL-1. Keynote Lecture 1

DYNAMICS AND MECHANISM OF UVR8 PHOTORECEPTOR

Author: Dongping Zhong[1]
1) The Ohio State University

UVR8 (UV RESISTANCE LOCUS 8) proteins are a class of UV-B photoreceptors in high plants. UVR8 is a homodimer that dissociates into monomers upon UV-B irradiation (280 to 315 nm), which triggers various protective mechanisms against UV damages. Uniquely, UVR8 does not contain any external chromophores and utilizes the natural amino acid tryptophan (Trp) to perceive UV-B light. Each UVR8 monomer has 14 tryptophan residues. However, only the epicenter two Trp (W285 W233) residues are critical to the light-induced dimer-to-monomer transformation. Here, combining time-resolved spectroscopy and extensive site-directed mutations, we have revealed the entire dynamics of UV perception to lead to monomerization, including a series of critical dynamical processes of a striking energy-flow network, exciton charge separation and recombination, charge neutralization, salt-bridge zipping and protein solvation, providing a complete molecular picture of the initial biological function.

> KL-2. Keynote Lecture 2

LIGHT-INDUCED HYPERTHERMIA FOR ONCOLOGY AND DISINFECTION

Author: Romain Quidant[1,2]
1) ICFO-Institut de Ciencies Fotoniques 2) ICREA-Institució Catalana de Recerca i Estudis Avançats

Recent years have witnessed a growing interest in controlling temperature on the nanoscale motivated by applications to different fields, including information technology, chemistry and medicine. Under illumination at its plasmon resonance, a metal nanoparticle features enhanced light absorption, acting as an ideal nano-source of heat, remotely controllable by light. Such a powerful and flexible photothermal scheme sets the basis of the emerging and fast-growing field of *thermoplasmonics*. In this talk, we first briefly present the specificities of heat generation in metal nanoparticles compared to standard macroscopic heating. We then focus on two different biomedical applications, namely less-invasive cancer treatment and disinfection of surgical implants.

In the first application, PEG-coated gold nanorods (PEG-GNRs) are tail-injected into an orthoxenograph mouse model of clear cell renal cell carcinoma. Due to their small size, PEG-GNRs can penetrate through the leaky tumor neovasculatures and eventually accumulate in the cancer tissue. This accumulation is non-invasively monitored over time using diffuse optics. Local hyperthermia is then locally induced upon a suitable NIR laser illumination. We study the nature of the cancer tissue damage and demonstrate tumor shrinking.

The second application relates to the prevention of biofilm formation at the surface of surgical implants. In our experiment, a surgical mesh, used for hernia surgery, is coated with a high density of GNRs. We demonstrate that under suitable illumination parameters, bacteria adhesion is reduced preventing the biofilm to form.

> KL-3. Keynote Lecture 3

IMAGING PHOTOTOXICITY AND PHOTODAMAGE IN CELLS BY FLUORESCENCE MICROSCOPY

Author: Thomas Gensch[1]
1) Institute of Complex Systems 4 (ICS-4; Cellular Biophysics) Forschungszentrum Jülich

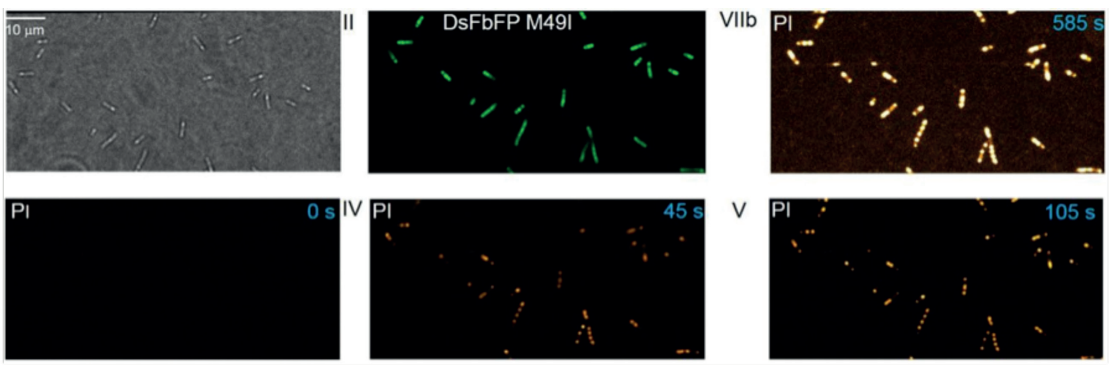
Content: Fluorescence microscopy and manipulation of cells, biological tissue and whole organisms have developed tremendously in the past 30 years. The photodynamic effect is among the most popular of these photonic manipulations with great relevance for therapeutic medical applications. Over the years many different and more and more sophisticated photosensitizing materials as well as ways for their incorporation and enrichment in cells and tissue of interest have been created and tested. In parallel, the rapid development of light sources, photodetectors, data recording, quality of optics, fluorophores, enabled the development of highly sophisticated methods that allow intelligent and minimal-invasive observation of living cells, tissue and organisms with unforeseen spatial and temporal resolution. In my lecture I will introduce a number of such fluorescence based microscopy methods, fluorescent sensors and photosenitizers and their application to visualize phototoxic species and effects, photodamage, apoptosis, necrosis and autophagy as well as cell parameters that report about healthy or abnormal state of cells like intracellular ion concentrations. Work in bacteria as well as mammalian cells will be presented.

Conflicts of Interest: TG declares that he has no conflict of interest.

References

1. M. Wingen, J. Potzkei, S. Endres, G. Casini, C. Rupprecht, C. Fahlke, U. Krauss, K.-E. Jaeger, T. Drepper, and T. Gensch. The photophysics of LOV-based fluorescent proteins – new tools for cell biology. *Photochemical and Photobiological Sciences* 13:875-883 (2014)
2. J. Torra, A. Burgos-Caminal, S. Endres, M. Wingen, T. Drepper, T. Gensch, R. Ruiz-González and S. Nonell. Singlet oxygen photosensitisation by the fluorescent protein Pp2FbFP L30M, a novel derivative of Pseudomonas putida flavin-binding Pp2FbFP. *Photochemical and Photobiological Sciences* 14:280-287 (2015)
3. S. Endres, M. Wingen, J. Torra, R. Ruiz-González, T. Polen, G. Bosio, N. L. Bitzenhofer, F. Hilgers, T. Gensch, S. Nonell, K.-E. Jaeger, and T. Drepper. An optogenetic toolbox of LOV-based photosensitizers for light-driven killing of bacteria. *Scientific Reports* 8:1502 (2018).
4. M.B. Rivas Aiello, D. Castrogiovanni, J. Parisi, J.C. Azcárate, F.S. García Einschlag, T. Gensch, G.N. Bosio, and D.O. Mártire. Photodynamic Therapy in HeLa Cells Incubated with Riboflavin and Pectin-coated Silver Nanoparticles. *Photochemistry and Photobiology* 94:1159-1166 (2018).
5. S. Burgstaller, H. Bischof, T. Gensch, S. Stryeck, B. Gottschalk, J. Ramadani-Muja, E. Eroglu, R. Rost, S. Balfanz, A. Baumann, M. Waldeck-Weiermair, J.C. Hay, T. Madl, W.F. Graier, and R. Malli. pH-Lemon, a fluorescent protein-based pH reporter for acidic compartments. *ACS Sensors* (2019) just accepted

Image:



Photodynamic action of flavin-binding protein DsFbFP M49I on *E.coli* bacteria (I: bright field image; II: fluorescence image) visualized by propidium iodide (PI) entering the damaged bacteria at different time points after excitation of DsFbFP M49I with blue light (III, IV, V, VIIb).

> KL-4. Keynote Lecture 4

SINGLET OXYGEN PHOTOPHYSICS: REVISITING THE PAST TO RECALIBRATE THE PRESENT AND REDEFINE THE FUTURE

Author: Peter R. Ogilby[1]
1) Department of Chemistry, Aarhus University

Singlet oxygen, $O_2(a^1\Delta_g)$, is a mature citizen in the disciplines of photophysics, photochemistry, and photobiology. Nevertheless, much remains to be learned about events that result in both the formation and removal of $O_2(a^1\Delta_g)$ in systems ranging from a neat liquid organic solvent to a functioning mammalian cell.

We have recently shown that $O_2(a^1\Delta_g)$ can be produced upon 765 nm irradiation of oxygen itself in sensitizer-free systems. With this tool in hand, I will summarize work we have done to (a) reevaluate the mechanism for solvent-mediated $O_2(a^1\Delta_g)$ deactivation, and (b) selectively produce $O_2(a^1\Delta_g)$ with subcellular spatial localization to induce, with forethought, either oxidative stress or eustress.

> KL-5. Keynote Lecture 5

X-RAY CRYSTALLOGRAPHY OF LIGHT-ACTIVATED PROTEINS – WATCHING THEM WORK AS A FUNCTION OF TIME OR LIGHT FLUENCE

Author: Antoine Royant[1,2]
1) Institut de Biologie Structurale, Grenoble 2) European Synchrotron Radiation Facility, Grenoble

Various types of steady state and time-resolved spectroscopies contribute to the understanding of the mechanism of light-activated proteins by probing the properties of their chromophore, which evolve upon changes in their chemical structure of the light-absorbing group or the influence of neighbouring residues. This local information is usually augmented by the availability of a protein structure, generally obtained by X-ray crystallography, whose output has surged over the last 25 years thanks to the multiplication of third-generation X-ray sources, synchrotrons. Most of these structures provide a static framework, in which one can put the extensive spectroscopic results in perspective. The field of time-resolved crystallography has developed at the same time, yet much slower, but ten years ago the advent of fourth-generation X-ray sources, free-electron lasers (XFELs), has given it a huge boost, while fostering new developments at synchrotrons. The structural changes in light-activated proteins can now be identified on the femtosecond to second time domains at XFELs (see [1] for structural changes ranging from nanoseconds to milliseconds). Slower time scales can now be routinely accessed at synchrotrons [2], from milliseconds to minutes. Finally, while deciphering the time evolution of structural changes is essential to the mechanistic elucidation, it may prove advantageous to study also the structural effects of increasing the light fluence on a protein crystal [3,4].

References

[1] Nango *et al.* (2016) *Science* **354**, 1552-1557
[2] Gotthard *et al.* (2019) *IUCrJ*, *in press*.
[3] Gotthard *et al.* (2017) *Biochemistry* **56**, 6418-6422.
[4] Torra *et al.* (2019) *Sci. Rep.* **9**, 2428.

> KL-6. Keynote Lecture 6

NEW LIGHT DEVICES FOR PHOTODYNAMIC THERAPY

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A homogeneous and reproducible fluence rate delivery during clinical PDT plays a determinant role in preventing under- or overtreatment. In Dermatology, topical PDT has been carried out with a wide variety of light sources delivering a broad range of light doses. However, these light sources do not deliver a uniform light distribution on the skin due to their structure and morphology and the complexities of the human anatomy. The development of a flexible light source able to generate uniform light on all its surface would considerably improve the homogeneity of light delivery. The integration of plastic optical fibers (POF) into textile structures offers an interesting alternative. The homogeneous light side-emission from the fabric is obtained by controlling the bending angles of POF inside the LEF due to specific architecture generated by knitting of textile structure. LEF of different surfaces can be easily manufactured (from 100cm² to 300cm²). The LEF thickness is less than 1 mm (1). The mean irradiance is typically *2.5* mW.cm^{-2}.W^{-1} with heterogeneity of 12.5 % at any point of the LEF. The temperature elevation remains below 1°C for a 45 minutes illumination (2). Similarly, Flexible Organic Light-Emitting Diodes have been recently evaluated for Antimicrobial Photodynamic Therapy. It has been shown that the OLED emission peak can be tuned from 665-725 nm to match the photosensitizer absorption range. Effectiveness was demonstrated on *S. aureus* using methylene blue as the photosensitizer (3). Multiple clinical studies have shown that interstitial photodynamic therapy (iPDT) is a promising modality in the treatment of cancerous tumors in prostate, pancreas, head and neck cancer and brain. The laser fibers are into the target tissue inserted via needles, or placed in catheters. However, the transport distance of light in biological tissues is limited by scattering and absorption. Practical therapeutic penetration depth is 0.1-1 mm for visible light in 400-600 nm and 2-3 mm for near infrared light in 700-1300 nm for most biological tissues. The fluence rate of therapeutic light must limited to prevent undesirable photothermal damage of tissues. The rate of oxygen consumption by the PDT process and the re-oxygenation rate of tissues may be also be an important consideration in deciding on fluence rate (4). Recently, several teams have elaborated innovative implantable and biodegradable light sources that can be used for iPDT and mPDT in particular. In contrast to conventional optical fibers, which must be removed from the body soon after use, the biodegradable and biocompatible light sources may be used for long-term light delivery and need not be removed as they are gradually resorbed by the tissue (5,6,7) .

> KL-7. Keynote Lecture 7

HOT TOPICS IN PHOTOPROTECTION

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Photoprotection is the most important strategy to prevent skin cancer and also all the deleterious effects of the excessive sun exposure. Wearing sun protective clothes and hats, reducing sun exposure in the midday and using sunscreens are the main behavioural measures, being the latest the predominant mode of sun protection. Even though in the last decades sunscreens have improved in terms of more acceptable vehicles and filters, there are controversies about their safety either for the environment or for the population. The possibility of differences between how sunscreens are tested under laboratory conditions and how they work in real life is an important issue that requires further investigation. In addition, the discovery of the effects of visible and infrared radiation on the skin and the possible interaction between these and ultraviolet radiation probably will require a more balanced photoprotection of the sunscreens for the different wavelengths. The use of new photoprotective molecules including antioxidants, DNA repairs or enhancers of the natural photoprotection of the skin will adapt photoprotection to individual needs towards a more physiological photoprotection.

SYMPOSIUM COMMUNICATIONS

PHOTOMEDICINE AND HUMAN PHOTOBIOLOGY

> IL001. Invited Lecture
Symposium MED-1 DNA-repair (Kaoru Sugasawa)

CHROMATIN DYNAMICS REGULATING RECOGNITION OF UV-INDUCED DNA PHOTOLESIONS

Authors: Masayuki Kusakabe[1], Erina Kakumu[1,2], Fumika Kurihara[1,2], Kanako Kusao[1,2], Kaoru Sugasawa[1,2]
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Ultraviolet light (UV) irradiation is one of the most common sources of DNA damage suffered from the environment. In many organisms including humans, nucleotide excision repair (NER) is exclusively responsible for removal of UV-induced photolesions from the genomic DNA. Unrepaired photolesions can cause genomic instability and/or cell death, and the hereditary defect in NER is implicated in several human recessive disorders associated with photosensitivity, such as xeroderma pigmentosum (XP).

In the human global genomic NER sub-pathway, DNA lesion recognition relies on two XP-related protein factors, XPC and UV-DDB. XPC senses the presence of disrupted or destabilized base pairs, rather than lesions per se, and thereby exhibits binding affinities not only for UV-induced photolesions, but also for bulky base adducts induced by numerous chemical compounds. In contrast, UV-DDB shows much more specific affinities for UV-induced photolesions and contributes to efficient recruitment of XPC to such lesion sites. Among UV-induced photolesions, cyclobutane pyrimidine dimers are processed mostly in a UV-DDB-dependent manner, whereas repair of pyrimidine (6-4) pyrimidone photoproducts can be initiated also through direct binding by XPC, in parallel with the UV-DDB-mediated lesion recognition pathway.

In living cells, DNA lesion recognition must be influenced profoundly by status of chromatin structures. When bound to lesion sites, UV-DDB seems to induce local decondensation of chromatin, which is probably advantageous to the subsequent recruitment of XPC. On the other hand, chromatin dynamics regulating the direct lesion recognition by XPC remain to be elucidated. We have reported that XPC directly interacts with histone H3, and this interaction is negatively regulated by acetylation of histone H3. Our results indicate that global hyperacetylation of chromatin (by inhibition of histone deacetylases) compromises recruitment of XPC to local UV damage sites, especially in the absence of UV-DDB. In this symposium, we would like to discuss unprecedented roles of histone modifications in regulation of DNA lesion recognition for global genomic NER, which may be intrinsically different from those in regulation of gene expression.

> IL002. Invited Lecture
Symposium MED-1 DNA-repair (Kaoru Sugasawa)

DNA DAMAGE DETECTION IN NUCLEOSOMES INVOLVES DNA REGISTER SHIFTING

Authors: Syota Matsumoto[1,2], Simone Cavadini[1,2], Richard D. Bunker[1,2], Ralph S. Grand[1,2], Alessandro Potenza[1,2,3], Julius Rabl[1,2], Junpei Yamamoto[4], Andreas D. Schenk[1,2], Dirk Schübeler[1,2], Shigenori Iwai[4], Kaoru Sugasawa[5], Hitoshi Kurumizaka[6,7], Nicolas H. Thomä[1,2]
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Access to DNA packaged in nucleosomes is critical for gene regulation, DNA replication and repair. In humans, the UV-DDB complex detects ultraviolet lightinduced pyrimidine dimers throughout the genome, yet it remains unknown how these lesions are recognised in chromatin, where nucleosomes restrict DNA access. Here we report cryo-electron microscopy structures for UV-DDB bound to nucleosomes bearing a 6-4 pyrimidine-pyrimidone dimer (6-4PP), and a DNA damage mimic at a variety of positions. We find that UV-DDB binds UVdamaged nucleosomes at lesions located in the solvent-facing minor groove without affecting the overall nucleosome architecture. For buried lesions facing the histone core, UV-DDB shifts the translational register of the DNA, moving the damage to an exposed position compatible with binding. These findings explain how UV-DDB detects occluded lesions in tightly positioned nucleosomes. We identify slide-assisted site-exposure (SAsSE) as a mechanism for high-affinity DNA-binding proteins to access otherwise occluded sites on nucleosomal DNA.

> IL003. Invited Lecture
Symposium MED-1 DNA-repair (Kaoru Sugasawa)

STRUCTURE AND MECHANISM OF PYRIMIDINE–PYRIMIDONE (6-4) PHOTOPRODUCT RECOGNITION BY THE Rad4/XPC NUCLEOTIDE EXCISION REPAIR COMPLEX

Authors: Jung Hyun Min[1], Debamita Paul[1], Hong Mu[2], Suse Broyde[2], Hong Zhao[3], Ouathek Ouerfelli[3], Philip Jeffrey[4]
Presenting Author: Jung Hyun Min
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Failure in repairing ultraviolet radiation-induced DNA damage can lead to mutations and cancer. Among UV-lesions, the pyrimidine-pyrimidone (6-4) photoproduct (6-4PP) is removed from the genome much faster than the cyclobutane pyrimidine dimer (CPD), owing to the more efficient recognition of 6-4PP by XPC-RAD23B, a key initiator of global-genome nucleotide excision repair. Here, we report a crystal structure of a Rad4-Rad23 (yeast XPC-Rad23B ortholog) bound to 6-4PP-containing DNA and 4-ms molecular dynamics (MD) simulations examining the initial binding of Rad4 to 6-4PP or CPD. This first structure of Rad4/XPC bound to a physiological substrate with matched DNA sequence shows that Rad4 flips out both 6-4PP-containing nucleotide pairs, forming an ‘open’ conformation. The MD trajectories detail how Rad4/XPC initiates ‘opening’ 6-4PP: Rad4 initially engages BHD2 to bend/untwist DNA from the minor groove, leading to unstacking and extrusion of the 6-4PP:AA nucleotide pairs towards the major groove. The 5’ partner adenine first flips out and is captured by a BHD2/3 groove, while the 3’ adenine extrudes episodically facilitating ensuing insertion of the BHD3 b-hairpin to open DNA as in the crystal structure. However, CPD resists such Rad4-induced structural distortions. Untwisting/bending from the minor groove may be a common way to interrogate DNA in NER.

> IL004. Invited Lecture
Symposium MED-1 DNA-repair (Kaoru Sugasawa)

THE XPA SCAFFOLD PROTEIN IN THE REPAIR OF UV-INDUCED DNA LESIONS BY HUMAN NUCLEOTIDE EXCISION REPAIR

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DNA repair pathways are essential to counteract the threat of endogenous and exogenous damage to DNA. The key pathway for the repair of UV-induced DNA adducts in DNA is nucleotide excision repair (NER). NER recognizes bulky DNA adducts and excises them from DNA by a stepwise and dynamic mechanism, involving six core factors, XPC-RAD23B, TFIIH, XPA, RPA, XPG and ERCC1-XPF and a number of additional proteins that facilitate NER in the context of chromatin. This presentation will illustrate our chemical and biological approaches toward understanding how protein-protein and protein-DNA interactions medite progression through the NER pathway. The focus will be on the XPA scaffold protein, which coordinates damage recognition with the dual incision to excise DNA lesions through interactions with the TFIIH, RPA and ERCC1-XPF protein. The importance of the XPA interaction network on the architecture of NER complexes and coordination of the various steps in NER will be discussed.

> IL005. Invited Lecture
Symposium MED-1 DNA-repair (Kaoru Sugasawa)

DETECTION OF THE sedDNA PRODUCTS OF NUCLEOTIDE EXCISION REPAIR IN UVB-IRRADIATED HUMAN SKIN

Authors: Michael Kemp[1]
Presenting Author: Michael Kemp
1) Wright State University

The exposure of human skin to UVB radiation results in the formation of photoproducts in epidermal cell genomic DNA that are solely repaired by the nucleotide excision repair (NER) system. Various methods have demonstrated that the NER machinery removes UV photoproducts from DNA in the form of small (~30-nt-long), excised, damage-containing DNA oligonucleotides in vitro (sedDNAs). Using surgically discarded human skin exposed to UVB radiation, the sedDNA products of NER were found to be readily detectable in small amounts of epidermal tissue ex vivo within minutes of exposure to sub-erythemal doses of UVB. Moreover, sedDNA generation was inhibited by treatment of skin explants with spironolactone, which depletes the epidermis of the essential NER protein XPB and thus mimics the skin of xeroderma pigmentosum patients. Analyses of sedDNA production in skin samples from differnet individuals revealed a wide range of inter-individual variation in NER activity. Together, these data suggest that sedDNA detection may be a useful assay for determining how genetic, environmental, and other factors influence NER activity in human skin epidermis

> IL006. Invited Lecture
Symposium MED-1 DNA-repair (Kaoru Sugasawa)

UBIQUITIN-MEDIATED REGULATION OF NUCLEOTIDE EXCISION REPAIR

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Nucleotide Excision repair (NER) removes a wide range of structurally unrelated DNA injuries that distort the DNA double helix, such as those induced by solar UV irradiation. NER is an essential DNA repair pathway to protect organisms against the severe consequences of DNA damage such as cancer and aging. The global genome NER (GG-NER) sub-pathway detects lesions in the entire genome and is initiated by the concerted activity of the UV-DDB and XPC protein complexes. XPC is the actual GG-NER initiator, capable of recognizing DNA helix-distortions induced by damaged DNA. The UV-DDB complex, consisting of the DDB1 and DDB2 proteins, associated to a larger E3 ubiquitin ligase complex, facilitates lesion recognition by XPC by binding directly to lesion that XPC cannot easily discriminate, such as UV-induced cyclobutane pyrimidine dimers (CPDs). Damage detection leads to the recruitment of transcription factor IIH (TFIIH), which unwinds DNA and verifies the presence of the lesion. Subsequently, the lesion is excised by the activity of the ERCC1/XPF and XPG endonucleases and the resulting gap is filled by DNA synthesis and ligation.

NER proceeds as a linearly ordered multistep process. Both damage detecting proteins for GG-NER, DDB2 and XPC, as part of UV-DDB and XPC-complex, are controlled by ubiquitylation, suggesting that their activities are tightly regulated. However, little is known about the dynamic interplay between the consecutive NER steps and how they feedback onto one another to assure efficient lesion repair. Our live cell imaging and biochemical data suggest that a tight ubiquitylation-mediated regulation of DDB2 levels at sites of damage controls its recruitment and dissociation. We found that this dynamic modification is necessary to ensure a smooth handover from DDB2 bound to DNA lesions to XPC, leading to the successive recruitment of TFIIH for damage verification. These studies illustrate that for efficient NER a well-organized, intricate and dynamic balance is required between damage detection and downstream NER complex assembly.

> IL007. Invited Lecture
Symposium MED-1 DNA-repair (Kaoru Sugasawa)

CONSEQUENCE OF CHRONIC LOW-DOSE UVB PRE-STIMULATION OF SKIN CELLS ON THEIR CAPACITY TO REPAIR CYCLOBUTANE PYRIMIDINE DIMERS

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Exposure to solar UVB leads to the formation of the highly mutagenic cyclobutane pyrimidine dimers (CPD), the DNA damage responsible for mutations found in skin cancers. The mutagenicity of CPD is caused, in part, by the fact that their recognition and repair by the nucleotide excision repair (NER) pathway is challenging and slow. It has been previously shown that a pre-stimulation with genotoxic agents improve NER efficiency of CPD, indicating a potential adaptive response of this repair pathway. We have pre-treated human diploid dermal fibroblasts and immortalized keratinocytes (HaCaT) with repeated subletal doses of UVB (chronic low-dose of UVB; CLUV) to determine whether it could enhance capacity to repair CPD. We have shown that CLUV treatment greatly enhance CPD repair and increases the level of NER recognition proteins, DDB2 and XPC, in fibroblasts. Surprisingly, the opposite has been found in HaCaT keratinocytes, i.e. an important decrease in CPD repair efficiency. In both cell types, CLUV irradiation leads to the accumulation of residual CPD that persist on DNA and are diluted via the semi-conservative replication. They are overrepresented in the heterochromatin and at the TT dipyrimidine sites, and they catalyze the incidence of sister chromatin exchange (SCE). Altogether, our results shed some light on the impact of chronic UVB exposure on repair and CPD accumulation.

> OC001. Oral Communication
Symposium MED-1 DNA-repair (Kaoru Sugasawa)

MOLECULAR MUTAGENICITY OF CYCLOBUTANE PYRIMIDINE DIMER IN MOUSE SKIN

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Cyclobutane pyrimidine dimer (CPD) is the main mutagenic photolesion among DNA damage produced by UVR. We reported the action spectra of UVR mutagenicity, namely the wavelength-dependent kinetics of mutation induction efficiency per UVR dose, previously [1], which were established using a large-scale, high-intensity monochromatic UVR source, Okazaki Large Spectrograph, placed in the National Institute for Basic Biology (Okazaki, Japan) [2] and a mouse strain transgenic with bacterial *lacZ* genes, which enables us to estimate mutagenicity *in vivo* in a specific organ such as skin epidermis and dermis in mice [3]. Now, we have established the action spectra of CPD formation in mouse epidermis and dermis, using the same UVR sources and mouse strain, along with the spectra of pyrimidine(6-4) pyrimidone photoproduct formation in the skin. Since a quantitative ELISA method has been introduced for the evaluation of photolesions [4], we have been able to estimate the efficiencies of photolesion formation on a molecular basis. Using these action spectra, we confirmed that UVR mutation occurs mostly depending on CPD formation. An analysis combining the action spectra of CPD formation and mutagenicity revealed that CPD mutagenicity, the mutagenicity of a CPD molecule, varies depending on wavelength and tissue. More detailed information will be given in our presentation.

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References

[1] H. Ikehata *et al.*, *J. Invest. Dermatol.*, 2013, **133**, 1850–6.
[2] M. Watanabe *et al.*, *Photochem. Photobiol.*, 1982, **36**, 491–8.
[3] J. A. Gossen *et al.*, *Proc. Natl Acad. Sci. USA*, 1989, **86**, 7971–5.
[4] H. Ikehata *et al.*, *Photochem. Photobiol. Sci.*, 2018, **17**, 404–13.

> OC002. Oral Communication
Symposium MED-1 DNA-repair (Kaoru Sugasawa)

INSIGHTS OF AN ANTARCTIC CLASS II CPD-PHOTOLYASE FROM THE UVC-RESISTANT STRAIN SPHINGOMONAS SP. UV9

Authors: Juan José Marizcurrena[1], Susana Castro-Sowinski[1]
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Ultraviolet (UV) irradiation produces inflammation, degenerative ageing and skin cancer. Among others, UVA/B causes direct and indirect DNA damage (oxidative stress and protein denaturation), meanwhile, UVC mainly causes direct DNA damage by formation of cyclobutane pyrimidine dimers (CPD) and pyrimidine (6,4) photoproducts (6,4PP). The Nucleotide Excision Repair (NER) system repairs these lesions, but in addition to NER, bacteria, fungi and bacteriophages produce glycosylases and enzymes of the Base Excision Repair that restore the damaged-DNA. However, the simplest way to repair a CPD or 6,4PP lesion is carried out by photolyases, enzymes that directly reverse this damage. Photolyases are found in all living forms, except placental mammals and some marsupials. Our hypothesis of work was that photolyases produced by UVC-resistant bacteria may efficiently fix the damage of human UV-irradiated DNA. A collection of Antarctic UVC-resistant bacteria was assessed and their UV-resistant behaviour was characterized. The microorganism with the highest photorepair potential, a bacterium from the genus *Sphingomonas*, was selected for genome sequencing. A total of two CPDs- and one 6,4-photolyase were found into the draft genome. Among them, a Cass II CPD-photolyase has been produced by DNA recombinant technology and purified to homogeneity. Homology modelling and sequence alignment analyses have shown this enzyme has a high similarity with the Class II CPD- photolyase from *Methanozarcina mazei* (2XRZ). *Methanozarcina* photolyase lacks an antenna chromophore and only needs a FAD cofactor for photorepair. Analysis by RMN and HPLC of our enzyme have shown only the presence of a FAD. The DNA-repair activity was analysed using a highly sensitive and specific monoclonal anti-CPD and anti-6,4PP antibodies (on UVC-irradiated calf thymus DNA; ELISA experiments) and also by HPLC (with irradiated-oligos). Results have shown that the recombinant photolyase fully repairs CPD-damaged calf thymus DNA and the synthetics oligos in both single and double-stranded DNA. Currently, we are analysing the repairing potential of 6,4PP, as we have seen repair of genomic DNA when using the ELISA assay, but we have not detected any repair ability of 6,4PP by HPLC when using oligos as substrate. Homology modelling and protein docking have also shown that our photolyase is able to bind both CPD and 6,4PP. Thus, this would be the first report of a photolyase able to repair both CPD and 6,4PP. Finally, we are prone to produce low-cost photolyases, that only requires a FAD cofactor to perform photorepair, with a high repairing potential of all direct UV-DNA damage. This enzyme may have cosmetic and pharmaceutical uses.

> IL008. Invited Lecture
Symposium MED-2 Ocular Photobiology (Joan Roberts)

REVIEW OF LIGHT DAMAGE TO THE HUMAN EYE

Authors: Professor Joan Roberts[Fordh]
Presenting Author: Professor Joan Roberts
1) Fordham University, New York

The human eye is constantly exposed to ambient radiation. Different wavelengths of light can be useful or damage compartments of the eye .

The primary factors which determine whether ambient radiation will injure the human eye are; the intensity of the light, the wavelength emitted and received by ocular tissues, and the age of the recipient. Corneal damage occurs with all radiation, while lens damage occurs with both UV-B above 295 nm and all of UV-A (295-400 nm). Adult retinal damage is usually caused by short blue light 400-450nm. By precisely defining and associating wavelength with a particular ocular disease, the wavelength (s) can be filtered by appropriate eye glasses and disease avoided or at least retarded.

Ocular photodamage may also be diminished by specific antioxidants and free radical quenchers. However, the underlying mechanism of protection must be exactly defined: singlet oxygen, peroxyradicals, hydroxyl radicals and other reactive oxygen species. Determining the specific reactive intermediate(s) produced by a particular phototoxic ocular chromophore not only defines the mechanism of toxicity but can also later be used as a tool to find specific antioxidant quenchers to prevent damage.

However, while quenching oxidative intermediates, the antioxidants themselves can be oxidized and must also be reduced.Without this appropriate combination of oxidizing and reducing agents, antioxidants become pro-oxidants and can potentially damage the eye and other organs as was found in the AREDS 1 clinical trial.

Understanding the proper use of supplements and filtering improper exposure to specific wavelenths of light directed toward the eye can significantly reduce or retard age related ocular disease.

> IL009. Invited Lecture

Symposium MED-2 Ocular Photobiology (Joan Roberts)

A COMPARISON OF THE ANTI-/PRO-OXIDATIVE MECHANISMS OF LUTEIN, ZEAXANTHIN AND OTHER CAROTENOIDS

Authors: Terence George Truscott[1], Ruth Edge[2], Fritz Boehm[3]
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It is well established that both zeaxanthin and lutein quench damaging singlet oxygen and that zeaxanthin is much more effective than lutein. However, several different types of reaction arise between dietary carotenoids and free radicals and this can lead to a switch from anti-oxidative to pro-oxidative processes. The reactions of xanthophylls (e.g. lutein and zeaxanthin) with several oxy-radicals will be reported. Time-resolved and steady state high energy (fast electron and gamma) radiation were used to generate and study a range of free radicals. These radicals included nitrogen dioxide ($NO_2^{\cdot}$), hydroxyl radical ($OH^{\cdot}$) and the superoxide radical ($HO_2^{\cdot}/O_2^{\cdot-}$). Nearly all strongly oxidising radicals, including $NO_2^{\cdot}$ (and, for example, $Br_2^{\cdot-}$ and $CCl_3O_2^{\cdot}$) react with carotenoids, via electron transfer, to 'quench' the free radical but also to generate the radical cation of the carotenoid. The one-electron oxidation potential of the carotenoid radical cations, so produced, is near 1000mV - they are strong oxidising radicals themselves. This can lead to a change from a protective role to pro-oxidative damage by dietary carotenoids. Also, processes other than electron transfer can arise. Most importantly, the hydroxy radical ($OH^{\cdot}$), despite being a strongly oxidising radical, adds to carotenoids rather than undergoing electron transfer and so generates a carotenoid free radical adduct (Car-$OH^{\cdot}$) rather than a carotenoid radical cation. These adducts can add molecular oxygen to give a carotenoid peroxyl radical (O_2-Car-$OH^{\cdot}$) -such species may well be damaging. Protective and damaging processes, of carotenoids with human lymphocyte cells will be discussed. In particular, the protection of human lymphocyte cells from $OH^{\cdot}$ damage is shown to be significantly dependent on the oxygen concentration – with efficient protection of the cells at 0% oxygen and virtually no protection at 100% oxygen. However, lutein (with only 10 C=C) shows the smallest 'oxygen concentration effect' leading to the best protection against $OH^{\cdot}$ compared to all the other carotenoids studied, including zeaxanthin, at atmospheric and higher oxygen levels. We suggest different roles for lutein and zeaxanthin, with zeaxanthin protecting against singlet oxygen and lutein protecting against peroxyl radicals.

> IL010. Invited Lecture
Symposium MED-2 Ocular Photobiology (Joan Roberts)

IN VITRO PHOTOTOXICITY OF RPE MELANOSOMES AND MELANOLIPOFUSCIN GRANULES FROM OLDER HUMAN DONORS

Authors: Tadeusz Sarna[Jagie], Magdalena Olchawa[Jagie], Justyna Furso[Jagie], Grzegorz Szewczyk[Jagie]
Presenting Author: Tadeusz Sarna
1) Jagiellonian University

Introduction:
Melanin in the human eye and skin is believed to play an important photoprotective role. However, melanin in the retinal pigment epithelium (RPE) does not undergo metabolic turnover serving its biological functions through lifetime. Being exposed to significant fluxes of visible light and high oxygen tension, RPE melanin could undergo substantial oxidative changes and modifications of its antioxidant and photoprotective abilities. The aim of this study was to analyze in vitro photoreactivity and phototoxicity of human melanosomes (MS) and melanolipofuscin granules (MLF) isolated from younger and older donors.

Methods:
ARPE-19 cells pre-loaded with MS or MLF, isolated from RPEs of 18-29 year old and 50-59 year old donors or MS and MLFG enriched with a combination of zeaxanthin and a-tocopherol (MS-A and MLFG-A), were irradiated with blue light for selected time intervals, and the cell survival was determined by MTT assay. Phagocytosis of FITC-labeled photoreceptor outer segments (POS) isolated from bovine retinas was analyzed by flow cytometry.The ability of MS and MLF to induce photooxidation of proteins was determined in a model system and in ARPE-19 cells using the fluorogenic probe coumarin boronic acid (CBA). Photogeneration of singlet oxygen by MS and MLF was measured by time-resolved near-infrared luminescence.

Results and discussion:
MS and MLF photogenerate singlet oxygen with the efficiency substantially increasing in the blue-violet part of the visible spectrum. Both pigment granules induce phototoxidation of bovine serum albumin and cellular proteins, with the effect being stronger for granules from older donors. The prooxidizing effect of MS-A and MLF-A is substantially reduced compared to granules not enriched with antioxidants. Irradiation of ARPE-19 cells containing MS and MLF with blue light reduces survival of the cells in a dose dependent manner. The observed phototoxicity is stronger for cells containing pigment granules from older donors, compared to younger donors and is weaker for cells with antioxidant-enriched pigment granules. ARPE-19 cells preloaded with pigment granules and irradiated with sublethal doses of light exhibit a transient inhibition of the specific phagocytic activity, which is more pronounced in case of MS and MLF from older donors. The phagocytosis inhibition is at least partially eradicated by supplementation of the pigment granules with antioxidants.

Conclusions:
Our study has demonstrated that one of the key functions of the RPE -- its specific phagocytosis -- can be impaired by photic stress mediated by the RPE melanosomes and melanolipofuscin granules. Aging may aggravate the inhibitory effect, which can be partially reversed by natural antioxidants such a zeaxanthin and a-tocopherol. The photoinduced inhibition of the phagocytic activity of RPE cell appears to be accompanied by oxidation of cellular proteins.

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> IL011. Invited Lecture
Symposium MED-2 Ocular Photobiology (Joan Roberts)

CORRECTING FOR COLOR BLINDNESS WITH ASSISTIVE FILTERS AND LIGHT SOURCES

Authors: Donald McPherson[1], Andrew Schmeder[1]
Presenting Author: Donald McPherson
1) EnChroma Inc.

Color Vision Deficiency, AKA Color Blindness affects more than 300 million people worldwide. Of this group, 4 out of 5 suffer from anomalous red-green color deficiency, a form that is addressable with EnChroma technology.

Normal and deficient color perception is described in terms of photopigment sensitivity. Light enters the eye, is captured by retinal photopigments and processed by Retinal Ganglion Cells (RGC) into channel information, which is then sent to the Lateral Geniculate Nucleus (LGN) for subsequent cortical processing. Action spectra of the photopigment classes determine the relative photon capture ratios and therefore channel values.

In the case of red-green anomalous color vision deficiency, excessive overlap of M- and L-cone action spectra leads to poor discrimination of colors that lie along confusion lines in color space. Type and severity of the defect determine the extent of color confusion. A basic assumption is that color confusion originates at the level of retinal photon capture, and that for the CVD all processes from the RGC through to the LGN, and then on to the vision centers in the occipital lobe with its myriad cortical processes are functional. Color confusion arises due to improper cone ratio signal. Providing corrected cone ratios allows cortical mechanisms to activate.

EnChroma manufactures lens and lighting technology that to some degree reestablish the correct photon capture ratio, thus leading to correct processing and perception of color-coded information. In the case of lens technology, EnChroma's filters and light sources selectively removes light from spectral region where maximal overlap of the photopigment action spectra occurs. In both lens and light technology, MM' and LL' photon capture is improved for the color defective towards normal ML ratios. The filters can be in the form of indoor and outdoor eyewear as well as contact lenses. EnChroma's assistive light source has application in learning and inspection environments.

A model of filter and lighting design is presented and is based on optimizing expansion of the color gamut. The product of light source, reflective surface and photopigments, from 400-700 nm at 1 nm intervals provides a baseline of data for normal and defective color vision, which can be represented in any number of standard color spaces. Introduction of the EnChroma filter in the above calculation shifts perception of hue and chroma selectively along red-green axis in color space while leaving the yellow-blue direction virtually unchanged. Optimizing the EnChroma filter over a large set of reflective surfaces leads to a set of filters that maximize color gamut for deuteranomal and protanomaly. Additional model constraints control the CRI and VLT for normal observers.

> IL012. Invited Lecture
Symposium MED-2 Ocular Photobiology (Joan Roberts)

OXIDIZED DOCOSAHEXAENOATE AS THE MAJOR PHOTOSENSITIZING COMPONENT OF RETINAL LIPOFUSCIN CONTRIBUTING TO LIPOFUSCIN FLUORESCENCE

Authors: Malgorzata Rozanowska[1], Anna Pawlak[2], Bartosz Rozanowski[3]
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Introduction
Docosahexaenoate (DHE) is an abundant retinal lipid present in high concentrations in retinal pigment epithelium (RPE) lipofuscin (LF). DHE and its enzymatic oxidation products play numerous important functions in the retina by providing fluidity to lipid membrane to enable effective signal transduction, including phototransduction, and acting as anti-apoptotic and anti-inflammatory agents. However, due to six unsaturated double bonds, DHE is extremely susceptible to non-enzymatic oxidation and forms numerous end-products, some of which have been identified in LF. During exposure of LF to light, its fluorescence changes. We hypothesized that oxidation products of DHE include fluorophores contributing to lipofuscin fluorescence and potent photosensitizers which can contribute to RPE dysfunction and toxicity upon exposure to light. The aim of this study was to compare fluorescent and photosensitizing properties of oxidized DHE (oxDHE) with LF.

Methods
LF was isolated from human cadaver RPE. Confluent ARPE-19 cells were fed daily for 13 days with LF followed by daily 1 hr exposures to visible light for 14 days. Cell viability and functions were monitored by morphology, attachment, endocytosis of neutral red, and mitochondrial activity. DHE was oxidized by exposure to the air at 37^{0}C and progress of oxidation was monitored by HPLC and absorption spectrophotometry. Photoreactivity of oxDHE was measured by electron spin resonance (ESR) oximetry and spin trapping with DMPO; and by time-resolved detection of electronically excited states, free radicals and singlet oxygen after laser flash photolysis. Fluorescence microscopy and spectrofluorometry were used to characterize LF fluorescence in RPE cells and oxDHE liposomes.

Results
DHE rapidly undergoes autooxidation and forms products absorbing visible light. Upon excitation of oxDHE with a 5 ns laser, a transient species is formed with absorption properties similar to RPE lipofuscin. Its quenching by oxygen and energy transfer to zeaxanthin, resulting in formation of zeaxanthin triplet state, identifies the species as an excited triplet state. Irradiation of oxDHE with visible light leads to the formation of superoxide and hydroxyl radicals. Photoexciation of oxDHE with a UV-A or blue light leads to formation of singlet oxygen quantum yields up to 24%. Similar to LF, the action spectra of light-induced oxidation of oxDHA show an increase in photooxidation with decreasing irradiation wavelength. LF-fed ARPE-19 cells exhibit golden-yellow fluorescence, which upon daily exposures to visible light gradually decreases while green fluorescence, similar to oxDHE increases. This change in fluorescence does not affect cell viability, mitochondrial nor endocytic activities.

Conclusions
Oxidized docosahexaenoate is the major photosensitizing component of retinal lipofuscin contributing to lipofuscin fluorescence.

Conflicts of Interest
None.

> IL013. Invited Lecture
Symposium MED-2 Ocular Photobiology (Joan Roberts)

PHOTOOXIDATION MEDIATED BY 11-CIS AND ALL-TRANS RETINAL IN SINGLE PHOTORECEPTORS

Authors: Yiannis Koutalos[1]
Presenting Author: Yiannis Koutalos
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Retinal, the vitamin A aldehyde, is a potent photosensitizer that plays a major role in light-induced damage to vertebrate photoreceptors. 11-*Cis* retinal is the light-sensitive chromophore of the vertebrate photoreceptor photopigment. It is isomerized by light to all-*trans*, activating the photopigment and beginning the process of light detection. All-*trans* retinal is then released by the activated photopigment, allowing the regeneration of the photopigment by fresh 11-*cis* retinal that is continually supplied to photoreceptors. The released all-*trans* retinal is reduced to all-*trans* retinol in a reaction using metabolic input in the form of NADPH. We have examined the photooxidation mediated by 11-*cis* and all-*trans* retinal in isolated living rod photoreceptors obtained from mouse, monkey, and human donor retinas. Photooxidation was measured as the lipid peroxidation induced by 360 nm light. Lipid peroxidation was measured with fluorescence imaging from the oxidation of internalized BODIPY C-11, a fluorescent dye whose fluorescence changes upon oxidation. The oxidation of BODIPY C-11 was measured from the shift in fluorescence between the intact (Ex: 555 nm; Em: 617 nm) and oxidized (Ex: 490 nm; Em: 528 nm) forms. We found that photooxidation increased with the concentration of exogenously added 11-*cis* or all-*trans* retinal to metabolically compromised rod outer segments that lacked an NADPH supply. Similarly, in dark-adapted metabolically compromised rod outer segments, photooxidation increased following exposure of the cell to light. However, in dark-adapted metabolically intact rod outer segments with access to NADPH, there was no significant increase in photooxidation following exposure of the cell to light. Finally, in metabolically intact human rod photoreceptor outer segments, there was no increase in photooxidation following addition of exogenous all-*trans* retinal, but there was significant increase following addition of exogenous 11-*cis* retinal. The results indicate that both 11-*cis* and all-*trans* retinal can mediate light-induced damage in rod photoreceptors. In metabolically intact cells however, the all-*trans* retinal generated by light is removed through reduction to all-*trans* retinol, minimizing any all-*trans*-retinal-mediated photooxidation. At the same time, and because the enzymatic machinery of the rod outer segment cannot remove 11-*cis* retinal, 11-*cis*-retinal-mediated photooxidation may play a significant role in light-induced damage to photoreceptor cells.

> IL014. Invited Lecture
Symposium MED-2 Ocular Photobiology (Joan Roberts)

ESTIMATING RETINAL EXPOSURES OF INTRINSICALLY PHOTOSENSITIVE RETINAL GANGLION CELLS (ipRGCs)

Authors: David Sliney[1]
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1) Consulting Medical Physicist

Because of changes in pupil size and upper-lid position in outdoor environments, light exposure of the paramacular area and the superior and inferior retina receive quite different exposure doses. The retinal field-of-view (FOV) cannot be neglected in quantifying the retinal irradiance of light stimuli during laboratory and field studies related to both light toxicity and non-visual effects related to intrinsically photosensitive retinal ganglion cells (ipRGCs). Although much emphasis has been placed on understanding the spectral variations of ipRGC responses and interactions with cone receptors, the spatial distribution also appears to be important. Since the ipRGCs are far less sensitive to light than the cones (by ~ 1000-fold), these melanopic receptors have been shown to be well functioning in daylight, but the ipRGCs along with the other photoreceptors also apparently play roles in providing the brain with indications of light level, time-of-day, transient adaptation, etc. The human retinal distribution of certain ipRGCs (e.g., M1 ganglion cells) and their spatial response differ depending upon retinal location, with the inferior retina apparently being more sensitive. These spatial variations may be more significant than previously thought.

> OC003. Oral Communication
Symposium MED-2 Ocular Photobiology (Joan Roberts)

ACUTE EPITHELIAL CELL DYNAMIC RESPONSES TO LOW DOSE UV RADIATION.

Authors: Naomi Delic[1,2], Nick Di Girolamo[3], Stephanie Watson[1,4], Gary Halliday[1], J. Guy Lyons[1,2,5]
Presenting Author: Guy Lyons
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Introduction
Along with the skin, the surface of the eye is the tissue most exposed to UV radiation from sunlight. The cornea provides most of the refractive power of the eye and maintaining its health and clarity are critical to maintaining high quality vision. The outermost cells, the corneal epithelium, form a barrier to protect the inner eye from physical and microbial damage and absorb much of the UVB from sunlight before it reaches the deeper layers of the eye. However, the effects of low doses of solar UV radiation, as would be encountered routinely by people, on the population dynamics and cell biology of the epithelial cells is poorly understood.

Methods
Corneas of fluorescent reporter strains of mice were imaged whilst alive by multiphoton microscopy, and post-mortem by super-resolution microscopy.

Results and Discussion
We recently showed that a low dose of UV (A+B) radiation, causing little cell death, causes a rapid increase in the turnover of the corneal epithelium on Confetti mice. This leads to a much faster migratory growth of epithelial clones from the stem cells, located in the limbus at the periphery of the cornea, to the centre of the cornea (Lobo *et al.*2016). Here, we demonstrate that the primary response site of UVR is the central cornea, not the limbus, and a single exposure has effects on the epithelium that last for 2 weeks. UVR increases the turnover of corneal epithelial cells by increasing the rate of delamination of cells from the basal layer of the epithelium. Quantitative analyses of LifeAct-GFP and E-cadherin-GFP mice show that this delamination occurs via a non-canonical form of cell extrusion, in which cells are pushed out of the basal layer by surrounding cells, but are retained on their apical surfaces. This loss of cells from the basal layer is compensated for by an increase in proliferation, particularly in the peripheral cornea and limbus.

Conclusions
UVR acts directly on the corneal epithelial cells and indirectly on their stem cell precursors to increase delamination and proliferation, respectively. Regulation of delamination from the basal layer by population pressure may be a general mechanism for regulating homeostasis and tissue damage responses in stratified epithelia.

Conflicts of Interest
None.

References

Lobo EP, Delic NC, Richardson A*et al.*(2016) Nat Commun 7, 12388

> OC004. Oral Communication

Symposium MED-2 Ocular Photobiology (Joan Roberts)

IN VITRO PHOTOTOXICITY OF RHODOPSIN PHOTOBLEACHING PRODUCTS IN THE RETINAL PIGMENT EPITHELIUM (RPE) AND THE EFFECT OF ANTIOXIDANTS

Authors: Magdalena Olchawa[1], Olga Krzysztynska-Kuleta[1,2], Krystian Mokrzynski[1], Tadeusz Sarna[1]
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Introduction

Although the primary biological function of retinal photoreceptors is to absorb light and provide visual information, extensive exposure to intense light could increase the risk of phototoxic reactions mediated by products of rhodopsin bleaching that might accumulate in photoreceptor outer segments (POS). Here we asked whether products of rhodopsin (Rh) photobleaching may contribute to dysfunction of the retinal pigment epithelium (RPE) under *in vitro* photic-stress conditions and whether selected flavonoids can modify phototoxic potential of Rh photobleaching products.

Methods

ARPE-19 cells or antioxidants enriched cultures were pre-loaded with Rh-rich POS isolated from bovine retinas and were irradiated with green light (520-580 nm, 7 mW/cm^2) to photobleach Rh, and subsequently with blue light (425 nm, 10 mW/cm^2) to excite retinoids, for selected time intervals. Survival of cells was determined by MTT assay and propidium iodide staining. Changes in mitochondrial membrane potential ($\Delta\Psi_m$) were assessed by JC-1 staining. Cells and model systems were also analyzed for the presence of protein hydroperoxides using the fluorogenic coumarin boronic acid (CBA) indicator. The effect of photic stress on specific and non-specific phagocytic activity of the cells was measured by flow cytometry.

Results

Irradiation or ARPE-19 cells containing phagocytized Rh-rich POS with green light and subsequently with blue light induced a weak dose dependent cytotoxicity accompanied by measurable reduction in cells mitochondrial membrane potential (MMP). Sub-lethal doses of PD-treatment mediated by rhodopsin-rich POS significantly inhibited the specific phagocytosis of POS and non-specific phagocytosis of polystyrene beads. In both cases inhibition of phagocytosis was transient and largely recoverable by 24 hours. Photic stress mediated by the rhodopsin-rich POS induced peroxidation of cellular proteins and bovine serum albumin (BSA) in model systems. Enrichment of cells with antioxidants lowered the detectable photoreactivity of Rh photobleaching products and reduced the inhibitory effect of retinoids mediated stress on POS phagocytosis.

Conclusions

The data support the hypothesis that products of Rh photobleaching, formed in POS, may also be present in RPE cells, where they could contribute to chronic oxidative stress and deterioration of the ability of RPE cells to phagocytize POS. Selected antioxidants may efficiently diminish the phototoxic action of retinoids, necessary for restoring the phagocytic function of ARPE-19 cells.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

> P001. Poster

Symposium MED-2 Ocular Photobiology (Joan Roberts)

ULTRAVIOLET RADIATION AS AN INITIATOR OF KERATOCONUS

Authors: Naomi Delic[1, 2], Stephanie Watson[3], Nick Di Girolamo[4], Gary Halliday[2], J. Guy Lyons[1,2]
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Introduction

Since it is located at the most anterior aspect of the eye, the cornea is subjected to significant amounts of ultraviolet radiation (UVR), resulting in a plethora of UVR-induced corneal conditions such as pterygia and ocular squamous cell carcinomas (SCCs). Keratoconus is characterized by stromal thinning and hence, protrusion or coning of the cornea that can often result in vision loss, but little is known about its mechanism of pathogenesis. Recently, a correlation has been made between geographical latitude and keratoconus prevalence, indicating that UVR exposure may play an important role in the development of this corneal affliction. The aim of this study was to establish a reliable method to model keratoconus and to determine whether UVR can contribute to the initiation and progression of the disease.

Methods

Mice were chronically exposed to low, physiologically relevant dose UVR, bi-weekly, and monitored for symptoms of keratoconus. Histochemical analysis of their corneas was performed after 9 and 20 weeks UVR to identify changes in their corneal architecture relevant to keratoconus.

Results and Discussion

This study found that chronic exposure to low dose UVR was sufficient to induce keratoconus-like symptoms in mouse corneas. After 9 weeks of chronic UVR, there was an increase in corneal curvature, a reduction in the number of epithelial layers and an increase in epithelial basement membrane fragmentation and collagen fibre disorganisation. After 20 weeks of chronic UVR, corneas displayed changes that were strongly representative of human keratoconus, such as epithelial and stromal thinning, loss of keratocytes, stromal cleft formation and fragmentation of the epithelial basement membrane.

Conclusions

Keratoconus is a multifactorial condition as shown by the heterogeneity of the disease. Here, we show the successful development of a model for studying keratoconus and that its initiation and progression likely the result of the accumulation of acute UVR effects from each exposure.

Conflicts of interest

None.

> P002. Poster

Symposium MED-2 Ocular Photobiology (Joan Roberts)

UV, RPE & AMD – THE EFFECTS OF ULTRAVIOLET RADIATION ON THE RETINAL PIGMENTED EPITHELIUM

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Background

Age-Related Macular Degeneration (AMD) is a leading cause of blindness in the western world. Recently, Recently, childhood ultraviolet radiation exposure (UVR) has been proposed as a possible risk factor, due to the increased UVR transmission of the young lens, however, epidemiological findings have been mixed.

Objective

To investigate the fundamental interaction between UVA/B radiation (290-400nm) and the retinal pigmented epithelium (RPE) within a controlled *in vitro* system to better understand its possible role in AMD.

Methods

By coupling a broadband source with narrow bandpass filters we were able to expose RPE cells, aRPE-19, to discrete 10nm bands of UVR between 290-400nm. Following exposure, we performed conventional biochemical assays (Prestoblue™) alongside electrical impedance spectroscopy (ECIS) and high-content imaging to quantify cell viability, mitochondrial stress, nuclear morphology, tight-junction integrity and oxidative stress. By tracking these parameters in relation to the irradiance of each wavelength we were able to produce action spectra for key parameters relevant to AMD.

Results

Using our unique approach, we have been able to identify discrete wavelengths of radiation, in the UV-A and UV-B bands, which RPE cells are acutely sensitive to in terms of cell viability, mitochondrial stress and tight junctional integrity.

Conclusion

In vivo, RPE cells are acutely sensitive to oxidative stress due to their high metabolic activity and oxygen perfusion. Our findings show that even in isolation of these factors, and accompanying age-related photosensitisers, RPE show particular sensitivity to discrete bands of UVR.

Future Work

Future work will focus on using iPSC-derived pigmented retinal tissue loaded with age-related photosensitisers, such as lipofuscin, which more closely models the cells found *in vivo* while leveraging high content imaging to quantify tissue parameters beyond viability and tight-junction integrity. Ultimately, these data will inform the use of action spectra to identify specific chromophores relevant to the pathology and prevention of AMD. Moreover, their weighting functions can be applied to global models of solar irradiance to highlight geographical regions of high AMD risk.

> P003. Poster

Symposium MED-2 Ocular Photobiology (Joan Roberts)

BLUE LIGHT INDUCED ACTIVATION OF MELANOPSIN SIGNALING PATHWAY IN HEK293 CELL LINE

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Introduction

Melanopsin is a member of the G-protein coupled receptors family. It is involved in non-image-forming responses to light including circadian rhythm, regulation of sleep, pupil response and other. Although significant efforts research have been devoted to different cell subtypes and their behavioral responses to light activation, signaling cascade involving melanopsin photoactivation is still poorly characterized. In this study, we analyzed the effect of photoactivation of melanopsin and different types of phospholipase C in HEK293 cells in vitro.

Methods

To determine the optimal condition of blue light exposure, survival of HEK293 cells expressing melanopsin was measured by MTT assay and image analysis of nuclear propidium iodide (PI) fluorescence at 0 hr and 24 hr after the cell irradiation. Real Time PCR measurements were carried out to investigate which type of phospholipase C is responsible for melanopsin activation, which was determined by mRNA level of FOS. The inhibition of PLC, induced by blue light irradiation, was analyzed by measurement of changes in concertation of intracellular calcium ions.

Results

Our result showed that only PLCβ_1 and PLCβ_4 subtypes were activated, by exposure of cells blue light, suggesting that only beta family PLC was involved in melanopsin signaling pathway. It was also observed, that treatment of the cell with PLC inhibitor -U73122, resulted in significant but not complete reduction of intracellular calcium level. Therefore the melanopsin signaling pathway is not limited to phospholipase C, but also another protein could be involved.

Conclusion

Phospholipase C plays a significant role in blue light activated melanopsin signaling pathway, both PLCβ_1 and PLC β_4 can be involved in this process.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest. The authors alone are responsible for the content.

> IL015. Invited Lecture

Symposium MED-3 Photoimmunology and photocarcinogenesis (Scott Byrne)

MECHANISMS BY WHICH NARROWBAND UVB PHOTOTHERAPY MAY REDUCE CONVERSION TO MULTIPLE SCLEROSIS BY PEOPLE WITH CLINICALLY ISOLATED SYNDROME, A PRE-FORM OF MS

Authors: Prue Hart[1], Stephanie Trend[1], Anderson Jones[1], Robyn Lucas[2], David Booth[3], Scott Byrne[3], Allan Kermode[4]
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1) Telethon Kids Institute, University of Western Australia, Perth, Australia 2) National Centre for Epidemiology & Population Health, Australian National University, Canberra, Australia 3) University of Sydney, Faculty of Medicine and Health, Westmead Institute for Medical Research, Westmead, Australia 4) Centre for Neuromuscular and Neurological Disorders, Perron Institute for Neurological and Translational Science, University of Western Australia, Perth, Australia

Introduction

Clinically isolated syndrome (CIS) is the earliest clinical episode in multiple sclerosis (MS). Low environmental exposure to UV radiation is implicated in risk of developing MS, and therefore, narrowband UVB phototherapy may delay progression to MS in people with CIS. Twenty individuals with CIS were recruited, and half were randomised to receive 24 sessions of narrowband UVB phototherapy over a period of 8 weeks. After 12 months, 7/10 of those receiving narrowband UVB phototherapy had progressed to MS whilst 10/10 in the no-phototherapy group had progressed (ref 1).

Methods

Peripheral blood samples for all participants were collected at baseline, and 1, 2, 3, 6 and 12 months after recruitment. An extensive panel of leukocyte populations, including subsets of T cells, B cells, monocytes, dendritic cells, and natural killer cells were examined in phototherapy-treated and control participants, and immunoglobulin as well as 25(OH)vitamin D levels measured in serum.

Results and Discussion

Conflicting with our initial hypothesis, there were no significant changes in Tregulatory cells in phototherapy-treated participants. There were significant short-term increases in the frequency of naïve B cells and intermediate monocytes, as well as reduced concentrations of switched memory B cells and classical monocytes in phototherapy-treated individuals. There was reduced arginase mRNA expression after 3 months by blood cells from phototherapy-treated participants. Although phototherapy increased serum 25(OH) vitamin D levels, the changes in B cells and monocytes have not been associated previously with vitamin D supplementation.

Conclusions

Several changes in blood cell subsets have been detected in CIS people receiving narrowband UVB phototherapy. More functional analyses are required to link cell changes with a UVB-induced reduction in CIS to MS conversion. UVB-induced pathways independent of vitamin D have been implicated.

Reference

1. Hart et al., A randomised, controlled clinical trial of narrowband UVB phototherapy for clinically isolated syndrome: The PhoCIS study. *Mult. Scler. J. Exp. Transl. Clin.* **4**, 2055217318 d12, doi:10.1177/2055217318773112 (2018).

> IL016. Invited Lecture

Symposium MED-3 Photoimmunology and photocarcinogenesis (Scott Byrne)

IMMUNOLOGICAL MECHANISMS UNDERLYING SUCCESSFUL PHOTOTHERAPY

Authors: Peter Wolf[1]
Presenting Author: Peter Wolf
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The exact mechanisms behind the therapeutic benefit of phototherapy have not been fully clarified and surely depend on both the pathophysiology of the disease treated as well as the modality of phototherapy administered. However, extensive research over the years particularly in the field of photoimmunology has paved the way to a better understanding on how the different phototherapeutic modalities act. In particular, pro-apoptotic and immunosuppressive effects, alone or in combination, may be crucial for phototherapeutic efficacy. Mechanisms of UV-induced immunosuppression, such as decreased number and function of antigen presenting cells in the skin, induction and activation of immunosuppressive Tregs, and increased release of inhibitory cytokines, such as IL-10, may counteract immune activation underlying cutaneous diseases such as psoriasis, vitiligo, atopic dermatitis, and other inflammatory conditions. In cutaneous T cell lymphoma recent work has shown that phototherapy induces a shift in benign T cell populations from a Th2 to a Th1 profile, potentially restoring natural anti-tumor responses, resulting in regression of clinical disease. Benign T cells were found to be associated with the Th2-recruiting chemokine CCL18 before therapy and with the Th1-recruiting chemokines CXCL9, CXCL10, and CXCL11 after therapy, supporting a switch from Th2 to Th1 phenotype. In sum, phototherapy seems to balance the immune response, whereby the direction of shift depends form the baseline situation.

> IL017. Invited Lecture

Symposium MED-3 Photoimmunology and photocarcinogenesis (Scott Byrne)

WILL HOLIDAY EXPOSURE TO UVR CHANGE YOUR IMMUNITY?

Authors: Joanna Narbutt[1]
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Ultraviolet radiation(UVR) is a known factor to induce immunosuppression, develop skin cancers and photoaging. On the other hand it is often used in dermatologic approach to treat various immune-mediated skin diseases. The exposure to UVR leads to the generation of genetic mutations, can activate various growth factors and cytokine receptors on the surface of cells. The consequence of this phenomenon is the activation of various intercellular signaling pathways, induction, and the activation of transcriptional factors, especially protein-1 and NF-kB, and changes in gene transcription. In the lecture I will show the results obtained during our research within EU research projects, focusing on the results of the UVR exposure on a holiday exposure on serum vitamin D3 and cyclobutene pyrimidine dimers in people. Our results, as well as literature data give clear recommendation to rigorous photoprotection.

> IL018. Invited Lecture

Symposium MED-3 Photoimmunology and photocarcinogenesis (Scott Byrne)

IMMUNOMODULATION OF UV-INDUCED IMMUNE SUPPRESSION: THE IMPACT ON SQUAMOUS CELL CARCINOMA ESTABLISHMENT

Authors: James Wells[1], Xuzhi He[1], Jazmina Gonzalez-Cruz[1], Margaret Veitch[1], Zhen Zeng[1], Shannon Joseph[1], Fiona Simpson[1]
Presenting Author: James Wells
1) [1]The University of Queensland Diamantina Institute, Faculty of Medicine, The University of Queensland, Wooloongabba, Australia

Introduction

The incidence of squamous cell carcinoma (SCC) and other non-melanoma skin cancers increases with decreasing latitude as a consequence of repeated, long-term exposure to ultraviolet (UV) radiation from the sun. UV exerts many biological effects associated with the establishment of skin cancer, including the accumulation of DNA mutations and the suppression of the immune system. The function of antigen-specific effector CD8 T cells in particular, which play a critical role in the prevention and control of tumour growth, becomes suppressed following the emergence of UV-induced regulatory T cells. Therapeutic strategies designed to mitigate the immune suppressive effects of UV therefore, may harbour potential for the treatment of non-melanoma skin cancers. In this study, we examine whether the immunomodulation of UV-induced suppression in a murine model of SCC can lead to effective tumour control.

Methods

We analysed the impact of UV-irradiation on the number and phenotype of $CD4^+Foxp3^+$ regulatory T cells present in the blood, spleen, skin, and skin-draining lymph nodes. Next, we explored strategies to reverse UV-induced immune suppression (immunomodulation) using a model of ovalbumin-induced contact hypersensitivity (CHS). Finally, we established the UV-exposure conditions under which HPV38 E6E7 mice would permit the growth of an adoptively transferred regressor SCC cell line, and examined the impact of immunomodulation after UV-exposure but prior to SCC transfer, on the growth of SCC tumours.

Results and Discussion

Five consecutive days of UV treatment with 150mJ/cm^2 of UVB increased the abundance of $CD4^+Foxp3^+$ regulatory T cells present in the skin-draining lymph nodes, but not in the spleen, blood, or skin. This short-term UV treatment regimen did not affect expression of $FR4^+$ or $CTLA\text{-}4^+$ on regulatory T cells, however it did have an immune-suppressive effect on CD8 T cell-driven CHS responses. Immunomodulation with anti-FR4, anti-CTLA-4, or IL-12, all prevented the establishment of UV-induced suppression in the CHS model. HPV38 E6E7 mice permitted the growth of syngeneic SCC regressor tumours following 10 weeks of 5-consecutive days-per-week UV treatment, which coincided with a small but statistically-significant increase and decrease respectively, in the proportion of regulatory T cells in the blood found to express FR4 and CTLA-4. Studies investigating the impact of immunomodulation on SCC growth and survival are ongoing.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

> IL019. Invited Lecture

Symposium MED-3 Photoimmunology and photocarcinogenesis (Scott Byrne)

PROTECTION AGAINST UV-INDUCED IMMUNOSUPPRESSION AND CARCINOGENESIS BY ORAL TREATMENT WITH A PROBIOTIC MOLECULE

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Introduction

Ultraviolet (UV) radiation promotes direct DNA damage that leads to mutations and malignant transformation of skin cells. Even though the immune system can efficiently detect and eliminate tumor cells, after skin exposure to UV radiation a strong immunosuppressive state is established. Probiotic bacteria are well known for their beneficial effects on human health, mainly on the digestive system and mucosal immunity. However, it has been proved that probiotics can also have impact on skin immunity.

Our aim is to study the role of a surface probiotic molecule, lipoteichoic acid (LTA) from *Lactobacillus rhamnosus*, on UV-induced skin damage.

Methods

To address our aim, we use different mice strains to induced carcinogenesis or CHS reaction suppression. All animals were orally treated with LTA (100 µg) during and/or prior to their exposure to a UVB lamp.

Results

Our first experiment showed a reduction in UV-induced carcinogenesis in SKH:1 mice after a 6 month irradiation schedule, when animals were treated with LTA all along the procedure. This reduction correlated with an activation of the gut associated lymphoid tissue (GALT) and a transient increment of CD4 and CD8 T cells in the skin draining lymph nodes.

Using an oxazolone-induced CHS reaction, we could determine that oral LTA prevents UV-induced immunosuppression in C57BL/6 mice. This effect was mediated by a recovery on the number and activation state of dendritic cells in the lymph nodes of LTA-treated UV-exposed mice after the sensitization phase of the reaction. This recovery leads to an efficient activation of T cells in LTA-treated mice. These effects ultimately promote an adequate effector T cells' recruitment to the challenged ear, reestablishing a normal inflammatory response.

Finally, we determined the ability of oral LTA to activate anti-tumoral immunity once skin tumors were established. SKH:1 mice were chronically irradiated without any treatment and separated in two groups once tumors were developed in all of the animals. At that moment, UV irradiation was suspended. One group of animals was orally treated with PBS whereas the other one was LTA-treated. The treatment was successful in reducing the number and the size of the tumors, but this effect was lost after the suspension of the oral LTA administration.

Discussion and Conclusions

Stimulation of the GALT through isolated probiotic molecules, such as LTA from *L. rhamnosus*, leads to changes in the immune system which reach the skin, preventing detrimental effects of UV radiation. Positive effects of oral LTA on skin immunity may be reversible, suggesting a role for innate immune mediators.

Acknowledgements

A. Friedrich, A. Larregina, J. Leoni, E. Cela, M.L. Paz, V. Campo and F. Weill.

Conflicts of Interest

None.

References

1- Br J Nutr. 2013 Feb 14;109(3):457-66. **2-** Int J Mol Sci. 2017 Jun 9;18(6). pii: E1067. **3-** Eur J Dermatol. 2008 Jul-Aug;18(4):476-7.

> IL020. Invited Lecture
Symposium MED-3 Photoimmunology and photocarcinogenesis (Scott Byrne)

PLATELET-ACTIVATING FACTOR AND UVB RESPONSES

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Platelet-activating Factor (PAF) is a family of bioactive glycerophosphocholines characterized by ability to act on the PAF receptor. Previously, our group and others have demonstrated that enzymatically produced PAF and PAF-like lipids produced non-enzymatically by reactive oxygen species are involved in UVB acute pro-inflammatory and delayed immunosuppressive effects. One important question in photobiology is how UVB, which just reaches the epidermis, can transmit systemic signals. Microvesicle particles (MVP) are small membrane bounded particles that carry bioactive molecules, such as proteins and nucleic acids and act as important signal transporters between cells. Previously our lab found that UVB and thermal burn injury can stimulate MVP release via PAF receptor activation and acid sphingomyelinase in epithelial cell lines and human skin. Skin keratinocytes released MVP can also activate PAF-R in recipient cells in response to UVB and thermal burn injury, indicating that PAF travels in MVP. Currently, studies from our group provide further evidence for a role of MVP in UVB-induced early inflammatory responses and delayed immunosuppression using murine models deficient in the PAF-R and acid sphingomyelinase, as well as mice treated with acid sphingomyelinase inhibitor. These studies suggest that MVP can serve as UVB effectors, in part via the ability to transfer the metabolically labile bioactive lipid PAF.

> IL021. Invited Lecture

Symposium MED-3 Photoimmunology and photocarcinogenesis (Scott Byrne)

EXPOSURE TO ULTRAVIOLET RADIATION ALTERS THE SKIN-DRAINING LYMPH NODE AND PLASMA LIPIDOME

Authors: Benita Tse[1], Anthony Don[1,2], Yen Chin Koay[3,4], John O'Sullivan[3,4], Scott Byrne[1]
Presenting Author: Scott Byrne
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Excessive exposure to ultraviolet radiation causes skin cancer but not getting enough UV is also associated with autoimmune diseases like multiple sclerosis. Suppression of the immune system is one of the events that links these detrimental and beneficial UV-effects. Previous studies have identified a key role for plateletactivating factor in mediating UVinduced immune suppression, but whether other UVinduced lipids are involved in immune suppression or whether changes in circulating lipids could act as a readout of "effective" UV exposure is not known. To assess this, we adopted a discovery lipidomics approach to identify novel molecules in plasma. Groups of C57BL/6 mice were exposed to an immune suppressive dose (8 J/cm^2) of solarsimulated UV. Blood was collected 24 hours later and plasma lipids analysed by discovery mass spectrometry. Seven unique circulating lipids were affected by UV exposure. Of these, 4 were within the same lipid family with similar fatty acid compositions. Importantly, these lipids were not murinespecific with all 7 lipids also being identified in normal human plasma. We also analysed the lipids within the skindraining lymph nodes as this is a major site where systemic immune suppression occurs. Although the 7 lipids identified in plasma were not altered in the lymph nodes, exposure to an immune suppressive dose of UV significantly affected 6 other lipids. Imaging mass spectrometry allowed us to pinpoint the anatomical location of these lipids and their relationship with lymph node cells. These studies are an important first step towards identifying clinicallyrelevant, novel biomarkers and mechanisms of lipid-driven UVimmune suppression.

> OC005. Oral Communication

Symposium MED-3 Photoimmunology and photocarcinogenesis (Scott Byrne)

ULTRAVIOLET RADIATION LOWERS BP IN A LARGE HAEMODIALYSIS COHORT

Authors: Richard Weller[1], Yuedong Wang[2], Jingyi Je[2], Franklin Maddux[3], Len Usvyat[3], Hanjie Wang[4], Martin Feelisch[5], Peter Kotanko[4]
Presenting Author: Richard Weller
1) University of Edinburgh 2) University of California, Santa Barbara 3) Fresenius Medical Care, North America 4) Renal Research Institute, New York 5) University of Southampton

Introduction

Hypertension is the leading global cause for premature death and disease. Most current treatment guidelines emphasize the importance of risk factors, but not all are known, modifiable or easily avoided. Population blood pressure correlates with latitude and is lower in summer than winter. Seasonal variations in sunlight exposure account for these differences, with temperature believed to be the main contributor. Vitamin D plays not part in blood pressure control. Recent research has indicated that ultraviolet (UV) light enhances nitric oxide availability by mobilising storage forms in the skin, suggesting that incident solar UV radiation may lower blood pressure. We tested this hypothesis by exploring the association between environmental UV exposure and systolic blood pressure (SBP) in a large cohort of chronic hemodialysis patients in whom SBP is determined regularly.

Methods

We studied 342,457 patients (36% Black, 64% White) at 2,178 U.S. dialysis centers over 3 years. Incident UV radiation/temperature data for each clinic location were retrieved from NOAA and NCAR databases. Linear mixed effects models with adjustment for ambient temperature, gender/age, BMI, serum Na^+/K^+ and other covariates were fitted to each location and combined estimates of associations calculated using the DerSimonian and Laird procedure.

Results

Pre-dialysis SBP varied by season and was ~4 mmHg higher in Black patients. Temperature, UVA and UVB were all linearly and inversely associated with SBP although the effect was more marked for UVB than UVA and in white than black patients. This relationship remained statistically significant after correcting for temperature although BP fell more for a given rise in irradiance at warmer temperatures.

Conclusions

In hemodialysis patients, in addition to environmental temperature, incident solar UV radiation is associated with lower SBP. This raises the possibility that lack/avoidance of sunlight is a new risk factor for hypertension and may account for reduced all-cause and cardiovascular mortality observed in more sun exposed individuals

> OC006. Oral Communication

Symposium MED-3 Photoimmunology and photocarcinogenesis (Scott Byrne)

UVR LIFE DOSE, SKIN PHOTOTYPE AND SKIN CANCER RISK – DETERMINED THROUGH THEIR COMMON RELATIONSHIP TO SOLAR LENTIGINES

Authors: Peter Alshede Philipsen[1], Ann-Sofie Sonne Holm-Schou[1], Luise Winkel Idorn[1], Elisabeth Thieden[1], Hans Christian Wulf[1]
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Background/purpose

Ultraviolet radiation (UVR) causes cutaneous solar lentigines and is the greatest individual risk factor for human skin cancer. The relationships between these lesions are complex and most likely include cumulative UVR dose, intermittent UVR exposures and skin phototype.

The aim was to investigate the association between lifetime UVR dose, skin phototype and skin cancer risk. As it is not practical possible to do a lifelong study we used their common relationship to solar lentigines.

Methods

This study investigated (i) the association between UVR dose and solar lentigines and (ii) the association between solar lentigines and skin cancer. By combining (i) and (ii) it is possible to estimate skin cancer risk related to UVR dose. Part (i) was based on longitudinal data (1999-2012) from 38 healthy participants using personal UVR dosimeters (a total of 16897 days with measurements) from which intermittent high dose sun exposure and individual lifetime UVR dose were estimated and related to facial solar lentigines assessed using black light photography. Part (ii) was based on a validated cross-sectional dataset of 2,898 participants including 149 participants with a skin cancer diagnosis. 116 had been diagnosed with BCC/SCC, 36 with CMM, and three of these with both. Their facial solar lentigines were assessed using black light photography, and skin phototype (pigment protection factor (PPF)) were objectively measured.

Results

In part (i), there was a borderline significant association (power function) between solar lentigines and lifetime UVR dose for men only ($p=0.060$). No significant association was found between solar lentigines and days with intermittent high dose sun exposure for men nor women ($p=0.626$). In part (ii), solar lentigines ($p<0.001$) and PPF ($p=0.001$) were significantly associated with skin cancer.

Combining part (i) and (ii) we found an increase in skin cancer risk of 1.23 by doubling the average lifetime UVR dose and the skin cancer risk was 34.9 times higher with a PPF of 1 (very fair skin) than with a PPF of 9 (dark Mediterranean skin).

Conclusion

It is possible to estimate skin cancer risk from estimated lifetime UVR dose and skin phototype. Skin phototype is of greater relative importance than lifetime UVR dose for skin cancer risk.

> OC007. Oral Communication

Symposium MED-3 Photoimmunology and photocarcinogenesis (Scott Byrne)

EPIGENETIC RESPONSE OF DIFFERENT cSCC (CUTANEOUS SQUAMOUS CELL CARCINOMA) CELL LINES TO UV IRRADIATION: IMPACT OF UV-RADIATION MODULATED miRNAs IN TUMOR PROGRESSION AND METASTASIS

Authors: Marc Bender[1], I-Peng Chen[1], Ivelina Spassova[2], Petra Boukamp[3], Peter Mohr[1], Beate Volkmer[1], Jürgen Becker[2], Rüdiger Greinert[1]
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Introduction

cSCC is the second frequent skin cancer worldwide. Although the main risk factor for its appearance is known – UV-radiation – the exact molecular mechanisms for development, progression and metastasis are still elusive. We therefore investigated the involvement of miRNA expression and the role of UV-radiation in tumor progression toward metastasis of cSCC. Profiling of miRNAs was performed in established cSCC cell lines obtained from a primary cSCC tumor (Met-1) or from a metastasis (Met-4) with or without UV-exposure to 3 different radiation qualities (UVA, UVB, UVA+UVB).

Method

MiRNA expression profiles have been screened with NanoString Assay (800 miRNAs) and further validated by flow cytometry (FirePlex, Abcam) and qPCR. Differentially expressed miRNAs both in Met-4 and irradiated Met-1 relative to unirradiated Met-1 cells were loaded into the Ingenuity Pathway Analysis tool (IPA, Qiagen) and a miRNA-gene interaction network was generated. Subsequently a pathway analysis to identify enriched pathways was conducted.

Results

A high number of differentially expressed miRs (17 down- and 22 up-regulated) was detected between the basal level of Met-4 (derived from a metastasis) and Met-1 using Nanostring assay. After UV-irradiation a dozen of miRs (e.g. miR-181a-3p) exhibits concordant changes in both Met-1 and Met-4 cell lines implying a common UV-response. On the other hand UV-irradiation also caused differential expression in either Met-1 or Met-4 only, suggesting cell line specific responses (e.g. let-7c-5p down-regulation after UVB only in Met-4). Despite the existence of UV-responsive miRs the PCA analysis revealed clear differences of the impact of cell origin as the first component accounting for about 50% of the differential miR expression. To access metastasis-associated miRs with regard to cSCC tumor progression and metastasis upon UV the miR expression profile of UV-irradiated Met-1 has been compared to that of unirradiated Met-4. Three miRs (miR-7-5p, miR-29a-3p and miR-183-5p) have been identified to be regulated commonly both in Met-1 after UV exposure and in Met-4 at the basal level, implicating involvement of these miRs in UV-induced cSCC metastasis. IPA analysis shows that miR-7, miR-29a and miR-183 build up a network encompassing possible targets which are known to be connected to skin cancer and metastasis e.g. PTEN, KLF4 and RAF1. Interestingly CNOT8 with deadenylation function is the only one gene targeted by all three identified miRNAs. Most important pathways which are connected to these miRNAs are e.g. UVB-Induced MAPK Signaling and Epithelial Adherens Junction Signaling. These results thus strongly suggest the functions of these 3 miRs in UV-triggered cSCC progression and metastasis.

> P004. Poster

Symposium MED-3 Photoimmunology and photocarcinogenesis (Scott Byrne)

IMPACT OF SOLAR UV RADIATION ON THE GENOTOXICITY OF POLYCYCLIC AROMATIC HYDROCARBON IN SKIN

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Skin is a major barrier against external insults and is exposed to combinations of chemical and/or physical toxic agents. Co-exposure to the carcinogenic polycyclic aromatic hydrocarbons (PAH) and solar UV radiation is highly relevant in human health, especially in occupational safety. *In vitro* studies on cultured cells have suggested that UVB enhances the genotoxicity of benzo[a]pyrene (B[a]P), the most carcinogenic PAH, by activating the AhR pathway and overexpressing the cytochrome P450 enzymes responsible for the conversion of B[a]P into DNA damaging metabolites. Our present work involves more realistic conditions, namely *ex vivo* human skin explants and simulated sunlight (SSL) as a UV source. As expected, we first observed that topically applied B[a]P strongly induced expression of cutaneous cytochrome P450 genes (CYP450 *1A1*, *1A2*, and *1B1)* and formation of DNA adducts to the diol-epoxide metabolite of B[a]P (BPDE). Interestingly, gene induction was significantly reduced when exposure to B[a]P was combined with SSL irradiation. Consequently, formation of BPDE-adducts was delayed when B[a]P exposure was associated with SSL irradiation performed either before or after. We then extended our work to more realistic PAH exposure by using organic extracts from real industrial samples, namely coal tar pitch. We used both a raw organic extract and a synthetic mixture mimicking the PAH fraction. We first observed that, although mixtures were very efficient at inducing expression of CYP450 *1A1*, *1A2*, and *1B1*, formation of BPDE adducts to DNA was drastically reduced as the complexity of the surrounding matrix increased. We then investigated the impact of simulated sunlight (SSL) on the effects of PAH in skin exposed to complex mixtures. Like upon co-exposure with pure B[a]P, SSL was found to decrease the expression of CYP450 genes when applied after and more efficiently before PAH treatment. Accordingly, the level of DNA-B[a]P adducts was reduced in skin samples exposed to both PAH and SSL. These results indicate that UV significantly impairs B[a]P and PAH metabolism, and decreases rather than increases immediate toxicity. The time-course observations made with B[a]P yet suggest that this phenomenon might be a delay rather than a complete reduction. It thus remains to clearly establish whether UV-induced decrease in metabolism efficiency may not change an acute exposure into a more chronic one as the result of an increased residence time of parent PAH in skin.

> IL025. Invited Lecture
Symposium MED-4 Photoaging (Rachel Watson)

THE PATHOPHYSIOLOGY OF PHOTODAMAGE

Authors: Rachel Watson[1]
Presenting Author: Rachel Watson
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Skin ageing is a complex process involving the convergence of two distinct mechanisms: the subtle effects of time-dependent intrinsic ageing, and the changes brought to bear on our skin by its constant interaction with the external environment, predominantly chronic sun exposure. This photodamaged skin has a distinctive clinical appearance, exhibiting coarse and fine wrinkles, a sallowness of complexion and reduced ability to recoil.

The early stages of photodamageing in lightly pigmented skin are characterised by degradation of some, but not all, components of the cutaneous elastic fibre system and by cumulative loss of fibrillar collagens, mediated by cellular pathways (matrix metalloproteinase and serine protease activity) and by direct photochemistry of proteins rich in ultraviolet-sensitive amino acod chromophores.

Functionally, extracellular matrix remodelling adversely impacts tissue behaviour, both mechanically and immunologically, and may further cause imbalance in the ability of cells resident within the tissue to signal to each other. Taken together, cutaneous photodamage results in significant loss of tissue function.

> IL024. Invited Lecture
Symposium MED-4 Photoaging (Rachel Watson)

UV-INDUCED PHOTODAMAGE IN SKIN OF COLOUR

Authors: Abigail Langton[1, 2]
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Photoageing describes complex cutaneous changes that occur over time due to chronic exposure to solar ultraviolet radiation superimposed on a background of intrinsic skin ageing. It occurs in habitually sun-exposed areas of the body such as face, neck, and arms and manifests clinically as wrinkles, lentigines, telangiectasia, mottled pigmentation, roughened texture and sallow complexion. Histologically, photoageing affects both the epidermis and dermis; epidermal changes include thinning of the spinous layer and flattening of the dermal-epidermal junction (DEJ). In the dermis, the most pronounced histological feature of photoageing is the accumulation of abnormally deposited amorphous elastin – termed solar elastosis – and disintegration of the well-organised elastic fibre network containing microfibrils rich in fibrillin and fibulin-5. At the functional level, photoaged skin exhibits increased laxity and decreased elasticity – biomechanical properties that are amenable to dynamic testing using non-invasive devices. These features of skin ageing are all applicable to lightly-pigmented skin (Fitzpatrick phototypes I-III); however, maintaining optimal skin function is essential for healthy ageing across global populations. In individuals with skin of colour (Fitzpatrick skin types IV–VI) ageing at photoexposed sites appears to manifest at a significantly slower rate and with less coarse wrinkling and laxity than is apparent in lightly-pigmented skin. With this in mind, the aims of the current study were: i) to characterize the biomechanical properties of young (n=21; 18-30 years) and aged (n=18; >65 years) photoexposed skin from cohorts of black African-American individuals and; ii) relate these biomechanical properties to the underlying architecture of the epidermis and organization of the dermis. Using non-invasive biomechanical testing we found that skin from young forearm is resilient (capable of returning to its original position following deformation); exhibits minimal fatigue; and is highly elastic. Histologically, young skin exhibits strong interdigitation of rete ridges and an abundance of fibrillar collagen and candelabra-like arrays of elastic fibres (fibrillin-rich microfibrils [FRM] and elastin). In chronically sun-exposed forearm, significant impairment of all biomechanical properties ($P<0.001$), complete flattening of rete ridges ($P<0.001$) and marked depletion of elastic fibres (FRM and elastin; $P<0.001$) and collagen I ($P<0.01$) prevail. We conclude that in skin of colour, despite the photoprotective properties of melanin, chronic sun-exposure significantly impacts skin function and composition. This study highlights the need for improved public health advice regarding the consequences of chronic photoexposure and the importance of multimodal photoprotection use for all regardless of ethnicity.

British Society for Investigative Dermatology 2019 Early Career Investigator.

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> IL023. Invited Lecture
Symposium MED-4 Photoaging (Rachel Watson)

COMBINED EXPOSURE TO UVA1 AND POLYCYCLIC AROMATIC HYDROCARBONS IMPAIRS HISTOLOGICAL QUALITY IN RECONSTRUCTED EPIDERMIS

Authors: Ariane Dimitrov[1], Martine Zanini[1], Charles Beauchêne[1], Jean-Philippe Belaïdi[1], Laurence Denat[1], Christophe Jones[1], Philippe Pérez[1], Olivia Zobiri[1], Sakina Mezzache[1], Dominique Erdmann[1], Joan Eilstein[1], Jérémie Soeur[1], Laurent Marrot[1]
Presenting Author: Ariane Dimitrov
1) L'Oréal Advanced Research

A previous clinical study showed a correlation between pollution, an altered barrier function and skin aging signs (pigmented spots) [Flament et al 2018]. Nanomolar concentrations of pollutants such as Polycyclic Aromatic Hydrocarbons (PAH, present in air, water or food [Farmer et al 2014]), have been detected in the blood [Song et al 2013] and within hair [Palazzi et al 2018] of individuals living in polluted areas. This suggests that deep skin may be contaminated by systemic exposure. The well-known photo-reactivity of some PAH upon UVA exposure could enhance their deleterious effects on skin [Wang et al 2005].

UVA1 (350-400nm) represents around 80% of d-UV (300-400nm) and penetrates deep into the skin, reaching dermis. Surprisingly, on 2D cultures of keratinocytes, UVA1 was associated with an equal or greater phototoxic effect than d-UV [Soeur et al 2017]. Associated with very low concentrations of PAH (nM), it impaired keratinocyte clonogenic potential at subtoxic doses and generated oxidative stress.

A multiple exposure protocol was developed to mimic a systemic chronic exposure on in vitro reconstructed epidermis with PAH and UVA1 at realistic doses. This treatment leads to a decrease of living epidermis thickness, to the appearance of morphological damages in the supra-basal layer and to the secretion of stress markers. These results suggest that epidermis renewal and differentiation could be impaired. Of note, Vitamin C can partially prevent these damages.

In such experimental conditions mimicking skin contamination in a polluted environment, our results suggest that chronic exposure to photo-polluting stress may impair cutaneous homeostasis.

> IL022. Invited Lecture
Symposium MED-4 Photoaging (Rachel Watson)

BENEFITS OF UVR EXPOSURE: STUDYING THE IMPACT OF AGE AND ETHNICITY ON CUTANEOUS VITAMIN D PRODUCTION

Authors: Mark Farrar[1], Richard Kift[1], Ann Webb[1], Lesley Rhodes[1]
Presenting Author: Mark Farrar
1) University of Manchester

Vitamin D is important for bone health and has been linked to many other health benefits including protection against a range of malignancies and autoimmune disorders. Our primary source of vitamin D is skin synthesis following exposure to UVB in sunlight. It is important to assess the vitamin D status of the general population in the context of recommended target levels and public health advice on vitamin D acquisition, which aims to balance the vitamin D benefit of sunlight exposure with the risk of skin cancer.

Through a series of longitudinal observation studies we have examined seasonal vitamin D status, personal UVR exposure, and time spent outdoors in population groups of differing age (12-15 years, 20-60 years, ≥65 years) and ethnicity (white Caucasian, South Asian). This has allowed the prevalence of vitamin D deficiency throughout the year to be determined for each group, and the relative impact of personal and behavioural factors on vitamin D status to be assessed.

Intervention studies have employed 6 week courses of low-level doses of UVR to simulate casual summer sunlight exposure. Thus, UVR-induced vitamin D production can be measured under identical protocols, exploring differences in age and ethnicity while controlling for behavioural factors. This allows the appropriateness of national guidance on sunlight exposure and vitamin D acquisition to be assessed for different population groups. Further intervention studies have examined the benefit-risk of UVR exposure through concurrent assessment of vitamin D production and DNA damage.

Data from observation and intervention studies have highlighted the need for more effective communication and targeting of guidance on the benefits and risks of sunlight exposure to the relevant population-groups.

> OC008. Oral Communication
Symposium MED-4 Photoaging (Rachel Watson)

INFRARED AND VISIBLE LIGHT IN SUN DAMAGE

Authors: Catherine Bonn[1], Rob Sayer[2]
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1) Newcastle University, Institute of Cellular Med, Dermatological Sciences, Newcastle Upon Tyne, England. 2) Croda Europe Ltd, Snaith, East Yorkshire, England

Introduction

Over a lifetime, we accumulate sun damage from UV radiation being absorbed by our skin. This leads to skin disease and premature ageing. But what about the visible and infrared light that comes from the sun? In this work, human cells and 3D skin models were exposed to wavebands of solar simulated light. UV on its own was found to cause damage, as were visible light and infrared light alone. In combination, all these wavelengths caused a damage response that was greater than the sum of its parts, suggesting a synergistic effect of UV, visible and infrared light.

Methods and Results

Reactive oxygen species generation was found to increase in primary cells (fibroblasts from the dermis and keratinocyte cells from the epidermis) when irradiated with solar-simulated UV. When fibroblasts were irradiated with all solar wavelengths concurrently, the response was higher than UV alone (72% increase in H_2O_2, $p < 0.05$). This increased damage in fibroblasts was also seen with both nuclear and mitochondrial DNA damage ($p < 0.001$). Keratinocytes did not show this pattern. This shows that dermal cells are more susceptible to the effects of visible and infrared light than epidermal cells.

Visible blue light was shown to increase radical generation in 3D skin models, examined using Electron Spin Resonance. Sunscreen formulations of spf 15 were found to reduce this radical generation in response to blue light alone, and to solar light containing UV, visible and infrared wavelengths. Formulations containing only UVB filters reduced radical generation by 20-31%, whereas formulations also containing UVA filters reduced radical generation by 70-75%, even when the cells were only exposed to visible blue light. This demonstrates that blue light could be contributing to sun damage, but that it is possible to protect against this damage with the appropriate sunscreen ingredients.

Discussion

Dermal fibroblasts had a higher level of damaging reactive oxygen species when exposed to all wavelengths of solar light together – UV, visible and infrared – compared to UV alone. This suggests that they are susceptible to damage by these wavelengths, while epidermal keratinocytes are not. As visible and infrared light penetrate through the skin to the dermis, this indicates that these wavelengths in sunlight may contribute to photoageing. This is supported by the evidence that blue light on its own can generate damage-inducing free radicals in 3D skin models. Fortunately, this can be reduced by using appropriate sunscreen ingredients.

> P005. Poster

Symposium MED-4 Photoaging (Rachel Watson)

ULTRAVIOLET RADIATION WAVELENGTHS CAUSING HARDENING AND REDUCED ELASTICITY OF COLLAGEN GELS AND EXCISED SKIN

Authors: Kazuhisa Maeda[1]
Presenting Author: Kazuhisa Maeda
1) Tokyo University of Technology

The skin is the largest organ in the body. It is located at the interphase between the external and internal environments and requires the development of efficient sensory and effector capabilities to differentially react to environmental changes. The dermis is partly composed of extracellular matrix components such as collagenous fibers, elastic fibers, and proteoglycans that contribute to skin elasticity and strength. Long-term exposure to ultraviolet (UV) B and UVA radiations in sunlight denatures the collagenous fibers in the dermis, which decreases skin elasticity and causes wrinkles. Immediate pigment darkening (IPD) and persistent pigment darkening (PPD) induced by UVA rays peaked at 340 nm and decreased gradually as the wavelength increased to 400 nm. However, whether the wavelengths that damage collagen are the same as those that cause IPD or PPD remain to be clarified yet. Therefore, the current standard in vivo test methods for UVA protective efficacy that use skin PPD as an indicator do not provide information on the effectiveness of UV protection products for the prevention of skin elasticity reduction.

The collagen fiber model and excised skin were exposed to UV rays, following which hardness and elasticity were evaluated and the wavelength spectra that caused the increased hardness and decreased elasticity were analyzed.

Radiation at wavelengths ranging from 300 to 340 nm caused hardening and reduced the elasticity of collagen gels and excised skin; radiation at 330 nm had the greatest effect. Hardening and elasticity reduction effects were not observed upon exposure of the collagen gels and excised skin to UV wavelengths of >350 nm. The UV rays that caused hardening and reduced elasticity of the collagen gels and excised skin differed from those of the PPD spectrum. PPD is used as a human protective factor against UVA radiation in sunscreen products. We found that UV radiation between 300 and 340 nm (UVB and UVA-II rays) caused these effects (with the maximum effect observed at 330 nm), while radiation at wavelengths ranging from 350 to 380 nm (UVA-I rays) did not have any effect.

> IL026. Invited Lecture
Symposium MED-5 Melanocytes and melanoma (Lionel Larue)

COMMON MECHANISMS REGULATING NEURAL CREST STEM CELL DEVELOPMENT AND MELANOMA FORMATION

Authors: Lukas Sommer[1]
Presenting Author: Lukas Sommer
1) University of Zurich

Tumor cells conceivably share properties with normal cells of the tissue, from which the tumor derives. Melanoma arises from the pigment cell lineage that originates during embryonic development from neural crest stem cells (NCSCs). Intriguingly, multipotent cells with NCSC properties have also been isolated both from normal adult neural crest target structures, such as the skin, as well as from human and mouse melanoma biopsies. Moreover, many factors known to regulate neural crest and melanocyte development also appear to be active and functionally important during melanoma formation. Interfering with features of normal NCSCs influences tumor growth and invasiveness both in genetic melanoma mouse models *in vivo* and in human melanoma cells. Likewise, a transcription factor signature active in NCSCs also appears to regulate 'stemness' properties in melanoma, and signaling pathways normally regulating NCSC fates control melanoma progression. Thus, developmental biology provides significant insights into the biology of melanoma.

> IL027. Invited Lecture
Symposium MED-5 Melanocytes and melanoma (Lionel Larue)

THE LOSS OF DICER AFFECTS THE INTEGRIN-MEDIATED MIGRATION OF MELANOCYTES AND THEIR HOMING IN THE HAIR BULB

Authors: Juliette Bertrand[1], Valerie Petit[1], Pierre Sohier[1], Franck Gesbert[1], Lionel Larue[1]
Presenting Author: Lionel Larue
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Age related hair greying is due to exhaustion of the melanocyte stem cells (McSC) pool. This phenomenon can be accelerated by genetic and/or environmental factors inducing stress and the premature death and/or early differentiation of McSCs in the bulge. Since Dicer is downregulated by stress, we inactivated this gene in the melanocyte lineage to investigate its contribution to McSC survival. The absence of Dicer in McSC at birth led to a progressive hair greying due to mis-localization and migration of melanocytes, and exhaustion of the McSC pool. An un-supervised approach revealed that mRNAs encoding integrins are enriched among the mRNAs modified by Dicer inactivation. More specifically, we showed that altered ItgaV and Itgb5 expression impacted melanocyte migration. Our data link Dicer, miRNAs (e.g., miR-92b), integrin expression (e.g., ItgaV) and Mc renewal. Altogether, we bring a novel cause of hair greying and its associated mechanism.

> IL028. Invited Lecture
Symposium MED-5 Melanocytes and melanoma (Lionel Larue)

THE FATE OF THE PIGMENT IN EPIDERMAL KERATINOCYTES: A CELL BIOLOGY POINT OF VIEW

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Skin color relies on mainly two cell types, the melanocytes and keratinocytes. In epidermis, the melanocytes form a membrane-enclosed organelle called the melanosome in which melanin is synthesized, stored and then transferred to the neighboring keratinocytes, the pigment-receiving cells. While melanocyte pigmentation and melanosome biology are quite well understood, how keratinocytes deal with the melanin are poorly defined. Given that the skin coloration and photo-protection is primarily due to the pigment residing in keratinocytes, there is a need to address the cellular and molecular processes underlying the entry, distribution and maintenance of the melanin in this recipient cell. I will discuss here about the in vivo distribution and packaging of the melanin in different human skin color types as well as the current development of a reliable in vitro system allowing to address the cell biology underlying the journey of the pigment in human epidermal keratinocytes.

> IL029. Invited Lecture
Symposium MED-5 Melanocytes and melanoma (Lionel Larue)

DEVELOPMENT OF A REPRODUCIBLE MODEL FOR STUDYING POST-INFLAMMATORY HYPERPIGMENTATION: EVIDENCE FOR A CAUSAL CONTRIBUTION OF AMBIENT LIGHT

Authors: Françoise Bernerd[1], Emilie Warrick[1], Claire Regazzetti[2], Christine Duval[1], Stéphanie Nouveau[1], Nathalie Cardot-Leccia[3], Virginie Piffaut[1], Thierry Passeron[4]
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Solar UV exposure is known to contribute to pigmentary disorders and recent data suggest a potential contribution for less energetic wavelengths (UVA, HEV). Post-inflammatory hyperpigmentation (PIH) is a very frequent pigmentary disorder, especially in pigmented skin individuals but its pathophysiology remains elusive especially due to lack of a relevant PIH model.

We developed an *in vivo* PIH model and assessed the role of low doses of ambient natural solar light in the commitment of the hyperpigmentary process. In melanocompetent volunteers, suction blisters were performed in the inner part of forearm. During wound healing process, suction blister areas were either protected with a total light block dressing (protected zone: PZ) or with a conventional one (unprotected zone: UPZ) allowing ambient light to reach the site. Colorimetric measures (ITA) and healing process were monitored. UPZ site was followed up to day 29 (D29) and a biopsy was taken. Other biopsies were performed in control, PZ and UPZ sites for transcriptomic analysis.

In all conditions, Trans Epidermal Water Loss normalization was observed at D9 and wound healing process was completed at D15. The pigmentation decreased during healing process and returned to basal at D9. A lightening effect was observed at D15 in the PZ area, while the UPZ site showed an induced pigmentation leading to visible hyperpigmentation at D29. This hyperpigmentation was histologically associated with epidermal hypermelanosis, pigmentary incontinence, vascular proliferation and polymorph inflammatory infiltrate. Tyrosinase and MITF stainings showed activated melanocytes with an increased dendricity. These features are hallmarks of PIH, similar to those observed in patients having PIH after inflammatory dermatosis. Interestingly, the PIH was prevented when the zone of the suction blister remained completely protected from all solar light radiation.

Transcriptomic analysis at D 9 and D15 in PZ and UPZ sites compared to a control zone, showed gene expression modulations related to healing process, with a D15 profile closer to that of the control site indicating the progressive completion of healing process. The differential analysis between PZ and UPZ conditions revealed at D15 only, a small set of modulated genes, which do not allow to highlight specific pathways as a signature of light exposure condition. Some genes related to epidermal biology were identified as well as the melanogenic gene PMEL17 which was statistically different between the two conditions. A targeted analysis of melanogenic related genes confirmed gene expression modulations for PMEL17, KITLG, TYR, TYRP1 between PZ and UPZ conditions, at D15, in line with the commitment of hyperpigmentation in UPZ site.

These results illustrate the development of a reproducible PIH model and the essential contribution of minimal amounts of ambient solar light in a context of healing process, inflammation and capillaries hyperplasia.

> IL030. Invited Lecture
Symposium MED-5 Melanocytes and melanoma (Lionel Larue)

THE MANY ROLES OF MITF IN MELANOCYTES AND MELANOMA

Authors: Eirikur Steingrimsson[1]
Presenting Author: Eirikur Steingrimsson
1) University of Iceland

The MITF transcription factor is essential for normal melanocyte development where it regulates proliferation, survival and differentiation. It also plays an important role in melanoma where it has been implicated in the switching of melanoma cells from proliferative, non-invasive cells to quiescent, invasive cells. How MITF has these different and sometimes contrasting effects is not fully understood. We have generated novel tools for characterizing the function of MITF in melanocytes and melanoma and have shown that it plays an important role in reorganizing the extracellular matrix and focal adhesions. This results in major effects on cell shape and interactions and may have implications for melanocyte migration and melanoma metastasis.

> IL031. Invited Lecture
Symposium MED-5 Melanocytes and melanoma (Lionel Larue)

THE GENETICS OF CONGENITAL MELANOCYTIC NAEVI

Authors: Veronica Kinsler[1,2]
Presenting Author: Veronica Kinsler
1) University College London Institute of Child Health 2) Great Ormond St Hospital for Children

Congenital melanocytic naevi result from *in utero* somatic mutations to the fetus, involving known oncogenes responsible for sporadic melanoma. The resultant phenotype depends not only on the gene involved, but on the timing of the mutation and the embryonic lineage and pluripotentiality of the cell hit. As with sporadic melanoma it is becoming increasingly apparent that the background germline genotype of the individual is also important in modifying the disease phenotype, and that this rare disease can be used to identify new melanoma predisposition genes. This lecture will review the latest knowledge of both somatic and germline genetics, and new data on potential mechanisms for predisposition.

> IL032. Invited Lecture
Symposium MED-5 Melanocytes and melanoma (Lionel Larue)

TELOMERE LENGTH, NAEVI AND MELANOMA

Authors: Veronique Bataille[1]
Presenting Author: Veronique Bataille
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In high risk melanoma families, individuals appear to be protected against photoageing with less solar elastosis, solar lentigines and solar keratoses than their age matched peers. This was a puzzling clinical observation as melanoma was previously linked to sun exposure so dermatologists led the way in assessing ageing phenotypes in patients at high risk of melanoma. In 2007, it was confirmed that this apparent delayed ageing was associated with longer white cell telomeres which reflects a delayed biological ageing. Number of naevi are also positively associated with telomere length. Polymorphisms (SNPs) in genes predicting white cell telomere length were then discovered and these SNPs were confirmed to be melanoma SNPs. Few rare melanoma families have now been found to have germline mutations in telomere genes whilst, at the somatic level, these mutations are commonly found in melanoma tumours. Telomere genes are currently included into melanoma gene panels for germline mutation screening and whilst mutations in genes such as the TERT promoter, TERT, POT1, ACD, TERF2IP and POLE are rare, they do occur in some very informative melanoma families especially in the presence of many other cancer primaries in the family. Colon cancer, glioma and chronic lymphocytic leukaemia have also been linked to POT1 and POLE mutations. This highlights the need to document all cancers in first and second degree relatives of melanoma patients as the clustering of other tumours in the family is important to assess if patients should be referred to a cancer genetic clinic for counselling and mutation screening. The discovery of these telomere genes also supports collaborations between cancer groups for advances in gene discovery as many genes involved in telomere maintenance are linked to many tumour types and not only melanoma.

> OC009. Oral Communication
Symposium MED-5 Melanocytes and melanoma (Lionel Larue)

SUNLIGHT: A DOUBLE-EDGED SWORD FOR MELANOMA?

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Presenting Author: Katie M. Dixon
1) Discipline of Anatomy and Histology, School of Medical Sciences and Bosch Institute, The University of Sydney, NSW 2006 Australia 2) Discipline of Physiology, School of Medical Sciences and Bosch Institute, The University of Sydney, NSW 2006 Australia

Melanoma is the most dangerous form of skin cancer, causing one death every five hours in Australia. A number of studies have linked vitamin D status to melanoma risk and outcome. Vitamin D is synthesised following UVB exposure of 7-dehydrocholesterol in skin cells, with eventual formation of the active metabolite 1,25-dihydroxyvitamin D3 (1,25D). Previous studies in our lab have shown that 1,25D (1-10 nM) can significantly reduce melanoma cell viability by targeting phosphatase and tensin homolog deleted on chromosome ten (PTEN), an inhibitor of the PI3K/AKT pathway. We now show that 1,25D can significantly ($p < 0.001$) reduce melanoma cell migration in a 3D *in vitro* model. N-myc downstream-regulated gene 1 (NDRG1) is a metastasis suppressor that is involved in many signaling pathways, including the PI3K/AKT pathway. Previous studies in our lab have demonstrated a significant ($p < 0.05$, $p < 0.01$) 1,25D-induced increase in NDRG1 levels in two melanoma cell lines. We now show the time course for this effect and furthermore demonstrate this in another five human melanoma (including metastatic) cell lines. Activity of 1,25D is usually mediated through the vitamin D receptor (VDR). We demonstrate that 1,25D can up-regulate NDRG1 either dependently or independently of the VDR to affect cell migration and angiogenesis. By up-regulating the metastasis suppressor NDRG1, 1,25D may act to inhibit melanoma metastasis, stop progression to angiogenesis and contribute to better outcomes for melanoma.

The authors declare no conflicts of interest.

> OC010. Oral Communication
Symposium MED-5 Melanocytes and melanoma (Lionel Larue)

VISIBLE LIGHT-INDUCED MELANOGENESIS IN HUMAN SKIN CAN BE REDUCED BY THE TYROSINASE INHIBITOR ISOBUTYLAMIDO-THIAZOLYL-RESORCINOL

Authors: Tobias Mann[1], Kerstin Eggers[1], Julia Riedel[1], Manuela Lütgens[1], Lisa Hemprich[1], Ludger Kolbe[1]
Presenting Author: Ludger Kolbe
1) Beiersdorf AG

The contribution of visible light (VIS) to skin pigmentation is well established. Recent studies showed that VIS, especially in the blue-violet range, significantly contribute to the darkening of melasma. However, further studies, analyzing light doses and intensities for induction of hyperpigmentation as well as new options for prevention and treatment are needed and, therefore, were the objective of our studies.

Solar simulators equipped with various filters to irradiate human skin with visible light in doses and intensities resembling one hour of mid-day summer sun in Central Europe were used in two in vivo studies and persistent pigment darkening after single irradiation and stimulation of melanogenesis after repetitive irradiation were measured. In one study the darkening of melasma spots after irradiation was monitored. In a second study, subjects were irradiated repetitively with VIS and treated daily with the tyrosinase inhibitor isobutylamido-thiazolyl-resorcinol. The influence on pigmentation was examined by clinical photography, clinical grading and spectroscopy.

Irradiation of melasma spots with VIS induced a persistent pigment darkening reaction which was still perceivable 24 hours after irradiation. Melasma spots darkened significantly stronger than irradiated adjacent normal skin. Repeated irradiation of normal skin with VIS stimulated long-lasting pigmentation which was reduced by treatment with the tyrosinase inhibitor isobutylamido-thiazolyl-resorcinol.

Visible light has a strong impact on human skin pigmentation, more than previously thought. Hyperpigmented spots react stronger to visible light than normal skin, thus, they were more perceivable after irradiation. Treatment of human skin with isobutylamido-thiazolyl-resorcinol reduced the VIS-induced skin darkening.

> IL033. Invited Lecture
Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

WHAT DO YOU REALLY KNOW ABOUT PHOTOPROTECTION?

Authors: Harvey Lui[1]
Presenting Author: Harvey Lui
1) University of British Columbia, Vancouver, Canada

Sun exposure must be approached with a “yin and yang” philosophy. The energy of the sun essentially drives all forms of terrestrial life on Earth, and yet the sun is the main cause of photoaging and skin cancer. There is now clear evidence that sunscreens not only prevent sunburns, but also certain forms of skin cancer and photoaging. Nevertheless, there are many myths and half-truths that continue to be advanced about sunscreens. In terms of the mechanism of action for sunscreens, it is important to realize that ALL sunscreens are actually “chemical” in nature, and they ALL work through “physical” processes. Furthermore dermatologists should understand and teach people that (1) sunscreens work immediately upon application, (2) reapplication every 2-3 hours is impractical for most people, and (3) there are many good reasons why we should be recommending sunscreens with SPF >30. Current commercial sunscreens do not provide adequate protection from the cutaneous effects of visible light. As compared to solar ultraviolet radiation, visible light has generally been considered physiologically inert, but there is now growing evidence that ambient visible light does indeed interact biologically with the skin, particularly in regards to pigmentation, and possibly even carcinogenesis and photoaging. Likewise, infrared radiation is now recognized to be important for exerting physiologic and pathologic effects on the skin. Finally, the public has grown increasingly skeptical of the personal and environmental safety of commercial sunscreens which in turn further compromises our ability to minimize harm from sun exposure.

> IL034. Invited Lecture
Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

THE ROLE OF POLYPODIUM LEUCOTOMOS IN PHOTOPROTECTION

Authors: Salvador González Rodríguez[Alcal]
Presenting Author: Salvador González Rodríguez
1) Medicine and Medical Specialties Department, Alcalá University

Increased exposure to sun leads to higher risk of sunburn, photoaging and skin cancer. In addition to topical barrier products, oral supplementation of various botanicals with endowed antioxidant and DNA repair activities are emerging as novel methods of photoprotection. A standardized aqueous extract of the fern *Polypodium Leucotomos* (Fernblock®) is powerful antioxidant due to its high content of phenolic compounds. This specific extract has been compared with several other commercialized extracts of *Polypodium Leucotomos* regarding antioxidant and photoprotective activities. This extract is safe when administered orally and can undergo topical absorption. Its mechanisms include predominantly reactive oxygen species (ROS) generation and release induced by ultraviolet (UV) light. It also prevents UV- and ROS-induced DNA damage with inhibition of both UV-induced AP1 and NF-kB activations, and protects natural antioxidant enzyme systems. At the cellular level, PL decreases cellular apoptosis and necrosis mediated UV and inhibits abnormal extracellular matrix remodeling. Fernblock activates tumor suppressor p53, inhibits UV-induced Cox-2 expression, reduces inflammation, and prevents immunosupresion. In agreement with increased p53 activity, this specific extract also decreased UV radiation-induced cell proliferation. PL also prevents UVA-induced common deletions mitochondrial DNA damage, and MMP 1 expression induced Visible Light and Infrared Radiation. These molecular and cellular effects may translate into long-term inhibition of photoaging and carcinogenesis.

> IL035. Invited Lecture
Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

BREAKTHROUGH IN PHOTOPROTECTION: TriAsorB, A NEW UV FILTER

Authors: Daniel Bacqueville[1]
Presenting Author: Daniel Bacqueville
1) Pierre Fabre Dermo-Cosmétique

Sun radiation plays a pivotal role in the development of actinic keratosis, skin cancers and photoaging. Photoprotection is thus a major issue in public health to prevent the harmful effects of solar ultraviolet (*UV*) radiations. Although various sunfilters are available worldwide on the cosmetic market, it remains important to improve consumer compliance and to develop innovative raw materials and novel formulation types with higher performance. In this context, our group Pierre Fabre Laboratories has developed a new UV filter named TriAsorB with the International Nomenclature of Cosmetic Ingredients (*INCI*) name Phenylene Bis-Diphenyltriazine.

TriAsorB has a high molecular weight of 540 g mol^{-1} (*CAS n°55514-22-2, EC 700-823-1,* $C_{36}H_{24}N_6$) and a Log $P_{o/w}$=10.5. It is a highly pure solid powder insoluble in a wide range of hydrophilic as well as lipophilic solvents. For an optimal efficacy of the UV filter, TriAsorB is ground to a specific particle size outside the nanoscale range (*beyond the threshold of 100 nm*). TriAsorB has been formulated as a cosmetic ingredient by wet grinding with an emulsifier (*PPG-1-PEG-9 lauryl glycol ether*), a preservative (*benzoic acid*) and water. The preparation is a 40-50% aqueous suspension of the finely dispersed active substance and is stable at room temperature for at least 18 months. It has a wide absorption spectrum in UVB and UVA *(λmax=355 nm,λc=384 nm, ε=52492 L* mol^{-1} cm^{-1}) and is not light sensitive (*greater than 98% according ICH Topic Q1B*). Thus, TriAsorB is a non-soluble broad spectrum UVB+A sunscreen. Spectrophotometric experiments have also shown that TriAsorB provides a protection in both the visible and the infrared spectral range, suggesting that TriAsorB is a full spectrum sunfilter. Furthermore, it has been able to protect a hair follicle-derived reconstructed human epidermis against the genotoxicity of simulated solar radiation.

Toxicological and bioavailability evaluations have been performed to evaluate the TriAsorB safety according to guidelines and/or Good Laboratory Practices as for example skin irritation/sensitization, repeated dose/reproductive toxicity and percutaneous absorption. All the data clearly demonstrated that TriAsorB is safe and calculation of margin of safety (*MOS*) was 980, a value largely superior to the recognized safety limit of MOS ≥ 100. Skin penetration studies showed that TriAsorB remained at the skin surface and did not diffuse into the skin. Recently, the European commission has approved the use of TriAsorB as a UV filter in final sunscreen products at a concentration of up to a maximum of 5% (*SCCS/1594/18*). Thus, TriAsorB presents no risk to human health and its use at a low concentration could also contribute to an environmental benefit.

Finally, TriAsorB represents a new generation of UV filter that might be used in combination with specific sunfilters in sun care products to afford skin photoprotection from UV to visible/infrared spectral range of the solar radiation.

> IL036. Invited Lecture
Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

PHOTOPROTECTION BY MYCOSPORINE-LIKE AMINO ACIDS (MAA)

Authors: Antony Young[1], Paul Long[1], Karl Lawrence[1]
Presenting Author: Antony Young
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Introduction

Solar exposure causes acute and long-term adverse effects in skin. These arise by direct and indirect damage to skin molecules, including DNA. Indirect damage is caused by the generation of reactive oxygen species (ROS). Topical sunscreens can prevent both acute and long-term consequences of solar exposure by attenuating solar UVR but are not known to have antioxidant effects. There is growing concern that synthetic sunscreen filters can damage the environment and possibly be harmful to humans. Indeed, 8 out of the 16 commonly used UVR filters currently licensed for use in the EU are now listed in the Community Rolling Action Plan (CoRAP) of the European Chemical Agency (ECHA) for safety evaluation. This has rekindled the search for safe biocompatible sunscreens. Mycosporine-like amino acids (MAA) are a family of >20 secondary metabolites commonly produced by marine plants that reside in shallow-water environments, which are typically exposed to high levels of solar UVR. Thus, they are believed to be natural sunscreens. By virtue of dietary accumulation from the marine food chain, MAA are also found in the sunlight-exposed tissues of some marine vertebrates, e.g. fish.

Results and Discussion

We demonstrate that MAA are highly effective in inhibiting a range of UVR induced damage in an *in vitro* skin model. Endpoints measured include DNA lesions (cyclobutane pyrimidine dimers (CPD), oxidative stress and gene expression changes associated with photoageing, inflammation and oxidative stress. We also show that MAA have several antioxidant properties, acting as chemical quenchers and biological antioxidants by activating the cytoprotective Nrf2 pathway. This work suggests that MAA may be developed as multifunctional photoprotective compounds, acting as photostable, biocompatible UVR filters with potent antioxidant properties. This is in contrast to current sunscreen filters that lack antioxidant capacity.

Acknowlegements

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References

• KP Lawrence, PF Long, AR Young (2018). Mycosporine-like amino acids for skin photoprotection. Curr Med Chem. 25:5512-5527.
• KP Lawrence, R Gacesa, PF Long, AR Young (2018). Molecular photoprotection of human keratinocytes in vitro by the naturally occurring mycosporine-like amino acid palythine. Br J Dermatol 178: 1353-1363.
• R Gacesa, KP Lawrence, ND Georgakopoulos, K Yabe, WC Dunlap, DJ Barlow, G Wells, AR Young, PF Long (2018). The mycosporine-like amino acids porphyra-334 and shinorine are antioxidants and direct antagonists of Keap1-Nrf2 binding. Biochimie. 154:35-44.

> IL037. Invited Lecture
Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

HOW CHINESE HERBS TO PREVENT AND REJUVENATE PHOTO-AGED SKIN

Authors: Leihong Flora Xiang[1], Chunyun Huang[1], Ye Liu[1], Li Chen[1]
Presenting Author: Leihong Flora Xiang
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Aging is a natural process leading to the progressive deterioration of the organs and its resultant clinical and histological changes. Photoaging is primarily due to solar ultraviolet (UV) radiation, which alters DNA, cellular antioxidant balance, signal transduction pathways, immunology, and the extracellular matrix (ECM). Photoaging is clinically characterized by coarse wrinkles, solar scars, roughness, dryness, laxity and pigmentation and is histologically characterized by disintegration of elastic fibers, degradation of collagen and thickened epidermal thickness. The photoaging is predominantly the effect of solar UV radiation that induces reactive oxygen species (ROS) and alters DNA/cellular homeostasis, which together alter signal transduction pathways and inflammatory cascade and induce immunosuppression and ECM remodeling.

Photoprotective strategies include blockade of UV photon Incidence, DNA repair, antioxidant activity, anti-Inflammatory effect, immunomodulation and regulation of ECM Remodeling. Extracts or isolated/purified substances from different parts of plants, including roots, leaves, flowers, seeds, etc., have been studies, and mainly function as antioxidants, also displaying anti-inflammatory and immunomodulatory activity and also modulating dermal ECM remodeling. Polyphenols are a large family of naturally occurring plant products that are widely distributed in plant foods, including fruits, vegetables, nuts, seeds, flowers and bark. The typical classification of those molecules takes into account the number and type of phenolics, which determine their biological properties. According to that, polyphenols are either flavonoids (the most numerous) or non-flavonoids, appearing in numerous plants.

According to the function of those natural ingredients, that could be summarized as follows.

Anti-oxidants: Green tea, grape seeds extracts (GSE), gynostemma pentaphylla (绞股蓝), Lucid Ganoderma(灵芝), Rhodiola Sachalinensis (红景天);

Anti-inflammation: Green tea;

Promoting collagen and fibroblast: Galla chinensis (GAC), Ginseng, Astragalus membranaceus (黄芪), Cordyceps polysaccharide (虫草多糖);

Removing or repairing UVB-induced DNA damage: Green tea, Ginseng, Paeoniflorin (芍药苷);

Immunoregulation: Royal Jelly;

Suppressing UVB-induced generation of ROS: Dalbergia odorifer (降香);

Most of these studies have been carried out in animal models using topical or systemic administration. I have no conflict of interest of this presentation.

> IL038. Invited Lecture
Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

TARGETED PHOTOPROTECTION: CAN SUNSCREENS BE ADAPTED FOR SPECIFIC USE IN DERMATOLOGICAL DISORDERS?

Authors: Giovanni Leone[1]
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Usually topical photoprotectors are dedicated to the prevention of skin damage caused by sun exposure. For this reason the main features that are taken into consideration are the protection factors (SPF and UVA PF) in order to guarantee the maximum protective effect against sun damage. Nevertheless there are some dermatological diseases in which a “blind” photoprotection may not be the best choice: in other words, providing maximum photoprotection does not correspond to an improvement of the disease, and, on the contrary, can worsen symptoms and interfere with common treatments. This is the case of vitiligo and psoriasis, two diseases in which moderate (vitiligo) or intense (psorisis) sun exposure can be of benefit. This is particularly true in the summer period when patients suspend phototherapy and saeek the positive effect of heliotherapy. We describe and discuss a new approach to photoprotection in these conditions where sun exposure may be part of the treatment. Dedicated sunscreens and correct education of the patients can represent a different approach that may help to control the disease, instead of “standard” photoprotection.

> IL039. Invited Lecture
Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

NICOTINAMIDE AND KERATINOCYTE CANCER

Authors: Diona Damian[1]
Presenting Author: Diona Damian
1) University of Sydney

Nicotinamide, a form of vitamin B3 and NAD precursor, has a range of in vitro and in vivo photoprotective effects. By preventing UV-induced ATP depletion, nicotinamide enhances the rate of DNA repair in human keratinocytes, melanocytes and in ex vivo UV-irradiated skin. Nicotinamide also attenuates UV-induced immune suppression in humans, when delivered topically or orally. In phase 2 and phase 3 studies in high risk patients, oral nicotinamide reduced actinic keratoses and also keratinocyte cancers (KCs; basal cell and squamous cell carcinomas). The ONTRAC study (Oral Nicotinamide To Reduce Actinic Cancer; Phase 3) included 386 immune competent participants with at least two KCs in the past 5 years. Patients were randomised to receive oral nicotinamide 500mg or placebo twice daily for 12 months and the primary endpoint was new histologically confirmed KCs during this time. Participants taking placebo developed an average of 2.4 new KCs in 12 months, compared to 1.8 new KCs in those taking nicotinamide [estimated relative rate reduction (RRR) 0.23 (95% CI: 0.04 to 0.38, p=0.02) adjusting for centre and number of KCs in the past 5 years]. Actinic keratoses were also significantly reduced by ~15% ($p<0.01$ compared to placebo at 3, 6, 9 and 12 months). We also used immunohistochemistry to examine immune cell infiltrates in and around the skin cancers arising on study with and without nicotinamide. Keratinocyte cancers arising in patients receiving nicotinamide showed significant reductions in $CD68^+$ macrophages but no differences in lymphocytes. The small number of melanomas arising on study showed in contrast significant reductions in CD4+ and CD8+ T cells. Nicotinamide is now a chemopreventive option for immune competent patients at high risk of skin cancer. Studies are now underway to assess the safety and efficacy of nicotinamide for chemoprevention in immune suppressed solid organ transplant recipients at extreme skin cancer risk.

> IL040. Invited Lecture
Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

WHAT ADVICE SHOULD WE GIVE OUR PATIENTS ON CONCERNS ABOUT ENVIRONMENTAL IMPACT OF UV FILTERS?

Authors: Henry W. Lim[1]
Presenting Author: Henry W. Lim
1) Department of Dermatology, Henry Ford Hospital, Detroit, Michigan, USA

The causal role of excessive exposure to artificial or natural UV light in the development of skin cancer is well-established. Photoprotection, which includes seeking shade when outdoor, wearing photoprotective clothing, hat and sunglasses, and applying broad spectrum sunscreen with SPF>30, is known to decrease the risk of skin cancer.

UV filters, especially oxybenzone and octinoxate, have been shown to have mild estrogenic effects in an animal model, and to bleach coral reefs in laboratory settings. This has led to the ban of oxybenzone and octinoxate containing sunscreens in Hawaii and Key West, Florida, starting in January 2021. Recent study also showed percutaneous absorption of UV filters in human subjects when sunscreens were applied at a maximum usage pattern (2 mg/cm^2, apply to 75% body surface every 2 hrs). These reports have led to significant confusion among the public.

Sunscreens have been in use since 1970s without any reported systemic side effects. Multiple studies have shown that ocean warming is the major cause of bleaching of coral reefs. As health care providers, we need to continue to emphasize to the public the health benefits of photoprotection, including the use of sunscreen in exposed area. For those concerned about the environment impact of organic (chemical) filters, inorganic or mineral filters (ie, titanium dioxide, zinc oxide) containing sunscreens can be used.

> OC011. Oral Communication
Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

UNCOVERING THE EFFICACY OF A NATURAL HOME-MADE SUNSCREEN ADVOCATED BY WELLNESS BLOGGERS ON SOCIAL MEDIA

Authors: Katie M. Dixon[1], K. Methmi M. Perera[1], Artur Shariev[1], Maria Byrne[1], Furkan A. Ince[1]
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In Australia, the use of approved sunscreens to protect against the damaging effects of ultraviolet radiation (UV) is strongly advocated by health authorities. Conversely, suggestions of harmful side-effects of sunscreen ingredients as well as effects on coral reefs by chemicals such as oxybenzone have prompted the search for alternative natural sunscreens. Despite the lack of scientific evidence supporting their efficacy, natural sunscreen recipes have been published widely by wellness bloggers on social media platforms and are growing in popularity. We tested the efficacy of a natural home-made sunscreen (NHSS) recipe promoted by a wellness blogger with 684,000 followers worldwide, aiming to provide some evidence to support or debunk such readily available online health information. The sunscreen contained (v/v) almond oil 39 %, coconut oil 19 %, shea butter 10%, beeswax 19 %, red raspberry seed oil 1.6 %, carrot seed oil 1.6 % and zinc oxide 5 %. *Ex vivo* human skin samples were obtained with consent from patients undergoing elective surgery. Skin sections were treated with either base lotion, NHSS or commercially available SPF50+ sunscreen (2 mg/cm^2) for 20 minutes prior to irradiation and during irradiation. NHSS was prepared either 3, 6 or 12 weeks prior to UV exposure and stored at room temperature in an opaque container. Skin samples were exposed to 20 J/cm^2 solar simulated UV. Three hours post-UV, skin samples were fixed and assessed for the level of UV-induced DNA damage in the form of cyclobutane pyrimidine dimers (CPDs) and 8-oxo-2'-deoxyguanosine (8oxodG) by immunohistochemistry, as well as sunburn cells 24 h post UV. Individual ingredients of this sunscreen were also investigated for their effects on UV-induced cell death and DNA damage in human dermal fibroblasts *in vitro*. Chemicals in sunscreens such as oxybenzone can be absorbed by corals and disrupt reproduction and growth cycles, eventually leading to bleaching. In light of this, we also investigated the effects of NHS in starfish embryo and larvae to determine whether the NHSS had any deleterious effects compared with SPF50+ sunscreen in this model. With online health information becoming increasingly popular through social media, the potential for conveying nonfactual advice is a concern. In this study, we uncovered the efficacy of a natural homemade sunscreen promoted by wellness bloggers on social media, and compared it to a commercially available SPF50+ sunscreen. We further examined the effects of both sunscreens in a marine ecosystem model to determine any potential effects on coral reef. Effective natural sunscreens that actually protect against the harmful effects of UV are likely to become increasingly popular given the recent drive to avoid chemicals that have been linked with side-effects and bleaching of coral reefs. It is therefore important to determine their efficacy before they are promoted to consumers.

> P006. Poster

Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

A BROADER FILTRATION OF UVA1 IMPROVES PHOTOPROTECTION: IN VITRO AND IN VIVO PROOF OF CONCEPT

Authors: Claire Marionnet[1], Christian Tran[1], Philippe Bastien[1], Anne Bielicki[1], Christelle Golebiewski[1], Diane-Lore Vieu[1], Didier Candau[2], Françoise Bernerd[1]
Presenting Author: Claire Marionnet
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Ultraviolet (UV) wavelengths reaching the Earth mostly include UVA1 rays (340-400 nm), that contribute to aging, immunosuppression and carcinogenesis in human (1-3). At the biological level, UVA1 rays induce epidermal and dermal skin damage, such as fibroblast apoptosis and alteration of the expression of genes and proteins, involved in essential pathways (4). However, today state of the art commercialized sunscreen formulas can efficiently filter UV wavelengths up to 370 nm, but lack sufficient absorption in the range of 370-400 nm UVA1 wavelengths.

We investigated if an enlargement of spectral absorption up to 400 nm would increase the protection against UVA1-induced damage in reconstructed skin *in vitro*, and in human skin *in vivo*. The efficiency of a state of the art formula (absorbing 280-370 nm rays), was compared with that of two prototype formulas. The latter were constituted of the state of the art formula to which prototype UVA1 filters were added, allowing a gain of absorption in the UVA1 range (up to 385 and 400 nm, respectively). *In vitro*, at differerent time points post UVA1 exposure, cell and tissue morphology, as well as gene expression (quantitative PCR) and soluble protein expression (ELISA), were evaluated after topical application onto reconstructed skin, prior exposure to UVA1. Different doses of UVA1 were assessed, ranging from 40 to 80 J/cm². *In vivo*, human skin darkening was assessed in 16 volunteers, skin type III/IV, using colorimetric measurements (Chromameter, Minolta CR300) and visual scoring, after topical application of the formulations on their back, followed by exposure to 50J/cm² UVA1.

In reconstructed skin, the use of formulas to which UVA1 filters were added, afforded a significant superior protection than the state of the art sunscreen, with regards to cell and tissue morphology, as well as gene and protein expression. *In vivo*, colorimetric measurements (ΔL*, ΔITA, ΔE) and visual scoring revealed that an enlargement of UVA1 spectral absorption up to 400 nm led to a significant better prevention of skin darkening, than a state of the art absorption profile. In *vitro* and in *vivo*, the broader the UVA1 absorption, the better the protection.

This proof of concept study demonstrated that an enlarged absorption profile in the UVA1 range improved the prevention of UVA1-induced epidermal and dermal damage *in vitro* and of skin darkening *in vivo*, compared to a state of the art profile of absorption. In line with other studies, this data pleas for a broader photoprotection in the UVA1 wavelengths domain.

References

1. Wang F, Smith NR, Tran BA, Kang S, Voorhees JJ, Fisher GJ. JAMA Dermatol 2014; **150**: 401-406.
2. Damian DL, Matthews YJ, Phan TA, Halliday GM. Br J Dermatol 2011; **164**: 657-659.
3. Tewari A, Grage MM, Harrison GI, Sarkany R, Young AR. Photochem Photobiol Sci 2013; **12**: 95-103.
4. Marionnet C, Pierrard C, Golebiewski C, Bernerd F. PLoS ONE 2014; **9**: e105263.

> P007. Poster

Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

ARE DIETARY CAROTENOIDS BENEFICIAL OR DELETERIOUS? THE OXYGEN EFFECT

Authors: Fritz Boehm[1], Ruth Edge[2], Terence George Truscott[3]
Presenting Author: Ruth Edge
1) Photobiology Research, IHZ 2) The University of Manchester 3) Keele University

Carotenoids are natural pigments, being constituents of a wide variety of fruits and vegetables, though chlorophyll often masks their presence. They are also responsible for the colouration of many flowers, birds and marine animals and some are essential to photosynthesis and vision. Believed to act as dietary antioxidants, having been shown to quench both singlet oxygen and a range of free radicals, carotenoids are of interest for their health benefits.

This study shows several dietary carotenoids protect against human lymphoid cell membrane damage from free radicals produced by ionising γ-radiation, and also by nitrogen dioxide generated photolytically. Blood was taken from volunteers who had supplemented their diet for 2 weeks with large doses of a specific carotenoid [70-90mg/day], or had minimized carotenoids in their diet. Radical-induced cell membrane destruction was shown by cell staining with eosin.

The carotenoid protective effect was reduced as oxygen concentration increased, particularly for damage due to γ-radiolysis. The effect is most pronounced for lycopene and β-carotene. For lycopene there is almost no protection under 100% oxygen, whereas there is 5-fold protection at 21% oxygen, and an extremely high, 50-fold, protection in the absence of oxygen. For β-carotene the values are observed to be 1.7-fold in oxygen, 5-fold in air and 44-fold without oxygen present.

This effect is less pronounced for nitrogen dioxide-induced cell killing, for lycopene, falling from 17-fold protection in the absence of oxygen to 9-fold at 100% oxygen.

Studies using the xanthophylls (carotenoids containing oxygen functional groups) have shown a reduced oxygen effect, for astaxanthin, zeaxanthin and lutein. In fact, lutein still imparts significant (25-fold) protection even under 100% oxygen conditions.

Gamma radiation cellular studies have also been undertaken with the addition of superoxide dismutase, proving that the oxygen effect is not due to reactions of the superoxide radical. Additionally, a series of non-cellular γ-radiolysis studies in simple solutions have also been carried out to help understand the molecular mechanisms for the oxygen effect.

The reduction in protection by carotenoids, particularly lycopene and β-carotene, at high oxygen concentrations may, perhaps, be one of the reasons why in lung cancer epidemiological trials, the β-carotene was shown to increase the number of tumours with statistical significance. However, the effect could possibly be exploited to enhance radiation procedures for therapy. Furthermore, the variation between the carotenoids indicates that supplementation with certain carotenoids may well be more suitable than others, for protection in environments with different partial pressures of oxygen.

> P008. Poster

Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

HEMEOXYGANASE-1: A VITAL PROTECTIVE ENZYME THAT INVOLVING IN ER STRESS AND Nrf-2 SIGNALING

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Ultraviolet A (UVA) irradiation is known as a double edged sword, it's not only a crucial environmental factor that contributes to inflammation, oxidation stress, and carcinogenesis, but also can be used as a phototherapy for skin diseases. In human HaCaT cell, we found that both HO-1 and HO-2 were silenced, UVA could lead to cell shrinkage, enhanced LDH leakage and increased cellular ROS level, in brief, we hypothesized that HO-1 and HO-2 are vital cyto-protective enzymes which could be target in genetic therapy for Ultraviolet protection.

UVA also cause endoplasmic reticulum stress, hence phosphorylates a subunit of eIF2. Meanwhile, UVA could also induce nuclear factor erythroid-derived two related factor 2 (Nrf2) and heme oxygenase-1 (HO-1), which belong to a vital protective signaling that against oxidative stress. In our latest research, we have found that UVA irradiation activated phosphorylation of eIF2a and Nrf2-HO-1 pathway in a dose-dependent manner. Modulation of eIF2a phosphorylation status with a selective inhibitor of eIF2a de-phosphorylation (Salubrinal) could alter expression pattern of Nrf2-HO-1 signaling and affect the cell cycle in mouse Keratinocyte cell line JB6. As a main sensor in the ER membrane, PERK could phosphorylate eIF2a resulting in ATF4 induced and ATF4 could also regulate HO-1. Meanwhile, PERK could also phosphorylate and activate Nrf2, which also provided us a new sight to explored the HO-1 transcriptional regulation under the crosstalk of ER stress and Nrf2 pathway. Besides, it also offered us to investigated the cascade regulation of HO-1 in response to different stimuli conditions.

> P009. Poster

Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

PROTECTIVE EFFECTS OF A CLEVER BOTANICAL COMBINATION ON HUMAN DERMAL FIBROBLASTS AGAINST BLUE LIGHT EMITTED FROM DIGITAL DEVICES

Authors: Azahara Rodríguez-Luna[1], Silvia Lorrio[2], María Gallego[2], María Gutiérrez-Pérez[2], Salvador González Rodríguez[3], Ángeles Juarranz[2]
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1) Medical Affairs Department Cantabria Labs 2) Biology Department, Sciences School, Universidad Autónoma de Madrid, Madrid, Spain 3) Medicine Department, Alcalá University, Madrid, Spain

Introduction

Nowadays the use of artificial visible light is on the rise and it is everywhere in modern life. We are highly exposed to artificial visible light, we can spend more than 6 hours per days in front of blue light (phones, tablets, computers, etc). The effects caused by solar exposure and the useful effects of visible light on the skin are well known. Nevertheless, the negative effects of visible light on skin are poorly known. Recent studies have shown that dermis is more affected than epidermis by blue light[1], which leads to activation of metalloproteinases, oxidative stress induction and long-lasting pigmentation[2]. In this line, we investigated the protective effect of an aqueous extract of *Deschampsia antarctica* (EDA) a polyextremophile plant which is able to thrive under tough environmental conditions, such as high solar irradiation or salinity[3], and an aqueous extract of *Polypodium leucotomos* (PLE) a naturally derived compound from fern's leaves native to South America[4]. PLE has been shown to have antioxidant and photoprotective properties. The aim of this study was to determinate if EDA, PLE and its combination could protect specifically against melanogenesis induced by blue light.

Methods

We evaluated EDA and PLE over human dermal fibroblasts (HDF) exposed to blue light. Visible source was a 400-500 nm LED lamp. Irradiation experiments were accomplished with a prototype of a narrow-band light-emitting diode (LED), which emits light of the wavelength λ = 450 nm. The light doses employed were 75,69 J/cm^2 and 151,38 J/cm^2. We evaluated cell viability by the MTT test, mitochondrial morphology by the fluorescence dye MitoTraker and the expression of mitogen-activated protein kinases p38 and ERK implicated in the melanogenesis pathway.

Results

In vitro studies have shown that blue light can promote cell death, mitochondrial damage and upregulation of ERK and p38 (which leads to up-regulated melanogenesis). However, we observed that pre-treatment with EDA, PLE and the combination of both could prevent cell death, mitochondrial morphology disruption and phosphorylation of mitogen-activated protein kinases (ERK y p38).

Conclusions

The use of this natural combination could prevent cell damage induced by blue light and long-lasting melanogenesis activation induced by blue light exposure. Taken together, all these results provide support to consider the application of this extract as a cosmetic approach against skin pigmentation encouraged by exposure to blue light from digital devices.

Acknowledgment

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Conflicts of Interest

A. R-L. belongs to Research and Development Department at Cantabria Labs and S.G. has a role of consultor for Cantabria Labs.

References

[1] Rascalou A. *J Dermatol Sci* 2018; **91**:195–205.;[2] Regazzetti C. *J Invest Dermatol* 2018; **138**:171–8;[3] Zamarrón A. *Int J Mol Sci* 2019; 1–18; [4] González S. *J Med Plant Res* 2018; **13**.

> P010. Poster

Symposium MED-6 Novel Forms of Photoprotection (Henry W. Lim)

PHOTOREACTIVE PROPERTIES OF NATURAL MELANIN PIGMENTS AND ITS EFFECT ON HaCaT CELLS

Authors: Krystian Mokrzy ski[1], Andrzej Dło[1], Grzegorz Szewczyk[1], Tadeusz Sarna[1], Michał Sarna[1]
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Introduction

Melanin pigments are produced in a multistage process by specialized cells such as the melanocytes. In the skin, melanin once produced is quickly transferred to neighbouring keratinocytes. Melanin, in particular, the brown-black eumelanin is viewed as an effective photoprotective agent against solar radiation due to its ability to absorb and dissipate light in the UV-vis range and scavenge reactive oxygen species. While eumelanin does it very efficiently, the yellow-reddish pheomelanin, on the other hand, seems to be less photoprotective and even photoreactive [1]. This is due to the fact that physicochemical properties of the two types of melanin differ significantly [2]. Recently, it was shown that pheomelanin can generate reactive oxygen species, in particular, singlet oxygen much more efficiently than eumelanin [3]. However, most of the studies done so far were made on synthetic models of melanin pigments. The lack of natural melanins to carry out such experiments leaves many questions unsolved. In this study, we examine photoreactive properties of melanins isolated from hair obtained from donors of different skin phototypes. We show that melanin from lighter skin is much more reactive than melanin from dark phototypes.

Methods

Photoreactivity and physicochemical properties of melanins obtained from donors of different skin phototypes were examined using a variety of different spectroscopy and microscopy methods such as: electron paramagnetic resonance (EPR) spectroscopy, dynamic light scattering (DLS), atomic force microscopy (AFM) and time-resolved singlet oxygen phosphorescence. Experiments on cells were conducted using human skin keratinocytes (HaCaT) cells. To examine the viability of the cells with phagocytized melanosomes MTT assay and propidium iodide (PI) fluorescence was employed. Cytoskeleton of the cells was analysed using confocal microscopy.

Results

The obtained results demonstrate that the enhanced photoreactivity of melanins from light skin individuals may lead to modifications of the cell mechanical properties, which are caused by changes in the cell cytoskeleton architecture.

Conclusions

Our work demonstrates that melanins differ in their photoreactive properties depending on the type of melanin.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

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References

[1] d'Ischia M et al. (2015) Melanins and melanogenesis: from pigment cells to human health and technological applications. Pigment Cell And Melanoma Research 28:520 – 544.
[2] Zadlo A et al. (2018) Photobleaching of pheomelanin increases its phototoxic potential: Physicochemical studies of synthetic pheomelanin subjected to aerobic photolysis. Pigment Cell And Melanoma Research 2018; 00: 1– 14. https://doi.org/10.1111/pcmr.12752
[3] Szewczyk G et al. (2016) Aerobic photoreactivity of synthetic eumelanins and pheomelanins: generation of singlet oxygen and superoxide anion. Pigment Cell And Melanoma Research 29:669 – 678.

> IL041. Invited Lecture
Symposium MED-7 Imaging (Harvey Lui)

CONFOCAL MICROSCOPY: WHERE ARE WE IN 2019?

Authors: Salvador González Rodríguez
Presenting Author: Salvador González Rodríguez
1) Medicine and Medical Specialties Department, Alcalá University, Madrid, Spain

Reflectance confocal microscopy´s journey from the lab prototype to the device in clinical practice is certainly a success story, but much remains to be done to achieve greater diffusion, facilitate training and widespread use in clinical practice. Its combination with dermoscopy has improved the accuracy of skin cancer diagnosis while reducing the number of biopsies of benign skin lesions. The most relevant confocal features of Non-Melanoma Skin cancers and Melanoma will be discussed including their correlation with routine histopathology. Finally, several clinical scenarios in which confocal microscopy is of great help for a better management in routine skin cancer clinics will be shown

> IL042. Invited Lecture
Symposium MED-7 Imaging (Harvey Lui)

CAN CONFOCAL MICROSCOPY PREDICT MOLECULAR BEHAVIOR IN SKIN TUMORS?

Authors: Francesca Farnetani[moden]
Presenting Author: Francesca Farnetani
1) Department of Dermatology

Cutaneous melanoma (CM) is one of the most prevalent skin cancers, which lacks both a prognostic marker and a specific and lasting treatment, due to the complexity of the disease and heterogeneity of patients. Reflectance confocal microscopy (RCM) in vivo analysis is a versatile approach offering immediate morphological information, enabling the identification of four primary cutaneous RCM CM types. RCM CM types correlated with markers of stemness property, density of intra-tumoral lymphocytic B infiltrate and cyclin D1 expression, while no significant association was found with blood vessel density nor molecular findings. RCM CM types show a different marker profile expression, suggestive of a progression and an increase in aggressiveness, according to RCM morphologies.

> IL043. Invited Lecture
Symposium MED-7 Imaging (Harvey Lui)

POLARIZATION SPECKLE AND IN VIVO SKIN APPLICATIONS

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1) BC Cancer 2) University of British Columbia 3) Vancouver Coastal Health Research Institute

Introduction
When laser light is directed to a skin surface, the backscattered light forms a stochastic interference pattern, which is called polarization speckle. The noisy and grainy pattern actually encodes surface and internal information of the imaged skin patch. Our research team has been investigating non-invasive techniques to image and quantify polarization speckle patterns in order to apply the techniques to human skin applications.

Methods
The principle of the techniques is based on the optical property of polarization, which describes the orientation of light wave oscillation. Technically, polarization is represented by a vector of four Stokes elements. The Stokes vector can be combined to derive other common polarization metrics, such as degree-of-polarization. In this line of research work, (1) we developed an *in vivo* method for measuring skin surface roughness. (2) In addition, we constructed a handheld polarization probe using low-cost optical parts with the goal that the probe could be widely used as a melanoma screening tool. To test the probe, we performed a pilot clinical study on 69 skin lesions including malignant melanoma (MM), squamous cell carcinoma (SCC), basal cell carcinoma (BCC), benign nevus (BN), actinic keratosis (AK), and seborrheic keratosis (SK). (3) We also investigated whether deep learning could improve the classification accuracy of skin cancer and common look-alike benign lesions.

Results
(1) Using the first two Stokes parameters, we developed an *in vivo* technique to quantify skin surface roughness expressed as root-mean-square roughness R_q. In a study of 27 males and 45 females with a mean age of 38 ± 14, we found that the body sites with habitually exposed to the sun were significantly more rough than the body sites received intermittently or minimal sun exposure. (2) In a pilot clinical study of 69 skin lesions, we found that the mean degree-of-polarization for MM (0.46 ± 0.09) measured *in vivo* by a polarization probe was significantly greater than that of other lesions (0.28 ± 0.01). (3) When we trained a 101-layer ResNet, a deep convolution neural network, to classify a set of malignant (423) and benign (679) polarization speckle skin images, the ResNet achieved an accuracy of over 80%, substantially higher than the accuracy of using a statistical method of mathematical moments.

Conclusions
We demonstrated that polarization speckle is potentially an effective optical tool for skin applications.

Acknowledgements
This line of work was supported in part by grants from Canadian Dermatology Foundation, Canadian Institutes of Health Research (CIHR), Natural Sciences and Engineering Research Council of Canada (NSERC), Vancouver General Hospital and UBC Hospital Foundation, and Vancouver Costal Hospital Research Institute.

Conflicts of Interest
The author holds a patent of the polarization technique and he is a board member of the International Society of Biophysics and Imaging of the Skin.

> IL044. Invited Lecture
Symposium MED-7 Imaging (Harvey Lui)

IN VIVO MULTIPHOTON MULTIMODALITY MICROSCOPY IMAGING AND MULTIPHOTON PHOTOTHERMOLYSIS THERAPY

Authors: Haishan Zeng[1,2]
Presenting Author: Haishan Zeng
1) University of British Columbia 2) BC Cancer Research Centre

We have developed a platform multimodality optical technology that integrated reflectance confocal microscopy (RCM) imaging, multiphoton microscopy (MPM) imaging, and confocal Raman spectroscopy (micro-Raman) for *in vivo* tissue analysis. In our system MPM further includes two imaging modalities: two-photon excitation fluorescence (TPF) imaging and second harmonic generation (SHG) imaging. RCM, TPF, and SHG images are acquired simultaneously in real-time and co-registered. Different modalities in the system provide complementary information. For example, when applied in non-invasive skin analysis, RCM visualizes cell boundary and intercellular structures, TPF visualizes cell cytoplasm and cell nucleus, while dermal collagen and elastin are well visualized by SHG and TPF respectively. Any interested microstructure identified by these imaging modalities can be measured with micro-Raman for biochemical information analysis. Application examples will be presented to demonstrate the powerful capability of this “super” system for skin diagnosis and analysis.

Based on the fact that multiphoton absorption occurs only at the focal point of a tightly focused femtosecond laser beam, we realized multiphoton absorption based photothermolysis in skin tissue utilized the above system with high illumination power. This multiphoton photothermolysis leads to highly spatially selective tissue damage with a precision of a few microns in size. Tissues in a micron size volume are damaged while the surrounding tissues are unaffected. An application example on closing single blood vessels in a mouse ear model will be presented. This precision therapy modality holds particular promise for treating diseases in complex organs such as the eye or brain, where high spatial selectivity is critical for preventing collateral effects on vision or central nervous system function.

> IL045. Invited Lecture
Symposium MED-7 Imaging (Harvey Lui)

NON-INVASIVE DETECTION OF SKIN CANCER USING RAMAN SPECTROSCOPY

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Skin cancer is the most common type of cancer. The current gold standard to diagnose skin cancer is through taking a skin biopsy, by excising skin tissue and then examination by a pathologist. Skin biopsies can be invasive, time consuming, costly and lead to complications. Further, for patients with multiple skin cancers this process can be tedious and not practical. Over recent years, there has been a great deal of research on using optical devices to detect skin cancer. Specifically in this presentation, the use of Raman spectroscopy to detect skin cancer will be explored. Raman spectroscopy captures unique optical signals via molecular vibrations in tissue samples. Our group initially was able to differentiate cancerous skin lesions with Raman spectroscopy based on the full band of Raman spectra, within the fingerprint region. The diagnostic accuracy of Raman spectroscopy to detect skin cancer is improved using wavenumber selection and by incorporating demographic patient details. Our Raman spectroscopy device provides an accurate diagnosis of skin cancer that is non-invasive and instantaneous.

> IL046. Invited Lecture
Symposium MED-7 Imaging (Harvey Lui)

MULTIMODAL OPTICAL EVALUATION OF VITILIGO

Authors: Harvey Lui[1], Jianhua Zhao[1], Sunil Kalia[1], Haishan Zeng[1]
Presenting Author: Harvey Lui
1) University of British Columbia. Department of Dermatology and Skin Science

Background
Reduced epidermal pigmentation is considered the primary pathophysiological difference between vitiligo and normal skin. Morphologic variants of vitiligo are based qualitatively on the distribution of lesions and their clinical appearances. The objective of this study is to quantitatively assess vitiligo in terms of its pathophysiological changes using multimodal spectroscopy.

Patients and Methods
Thirty-seven patients (17 male, 20 female) were recruited in this study with a mean age of 42 (range: 18-74) years, and covering skin types I (4), II (9), III (13), IV (7) and V (4). Lesions of vitiligo and the adjacent normal skin were measured using diffuse reflectance and Raman spectroscopy. Skin color was calculated from the diffuse reflectance spectrum in the CIE L*a*b* color space. Biophysical properties including melanin, oxy-hemoglobin and deoxy-hemoglobin and scattering were calculated using the empirical Kollias algorithm. Lesion versus normal properties were analyzed statistically using the two-tailed non-parametric paired Wilcoxon test.

Results
Vitiliginous skin exhibits significantly higher L*, lower a* and b* than adjacent normal skin ($p<0.0001$), indicating that affected skin appears relatively lighter, less yellow, and slightly less red. These color changes related to the underlying pathophysiological changes. Vitiligo lesion has much lower melanin content ($p<0.0001$), scattering ($p<0.0001$) and deoxy-hemoglobin ($p=0.0014$), but higher oxy-hemoglobin ($p<0.0001$) and oxygen saturation ($p<0.0001$). Biochemical changes in vitiligo were also identified by Raman spectroscopy where it was found that vitiligo lesions appear to have relatively higher keratin, collagen and hemoglobin signals, and lower signals for melanin, carotene and nucleic acids.

Conclusions
Multimodal spectroscopy reveals that difference between vitiligo and normal skin is more than melanin. Excessive oxy-hemoglobin and keratin signals may indicate localized inflammation; while excessive collagen and reduced carotene and nucleic acid signals may possibly indicate photodamage as a consequence of reduced melanin protection within vitiligo lesions

> OC012. Oral Communication
Symposium MED-7 Imaging (Harvey Lui)

LONGITUDINAL STUDY OF THE SKIN RESPONSES TO UVB CHALLENGES USING NON-INVASIVE MULTIMODALITY MICROSCOPY

Authors: Giselle (Yunxian) Tian[1,2], Harvey Lui[1,2], Jianhua Zhao[1,2], Zhenguo Wu[1,2], Sunil Kalia[1,3], Vincent Richer[1], InSeok Seo[4], Hao Ou-Yang[4], Haishan Zeng[1,2]
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Background
Serial analysis of cellular dynamics over time offers new insights into human skin responses to solar radiation. However, most of the previous studies are based on multiple biopsies and *ex vivo* analysis which precludes the monitoring of the same sites and cells over time. *In vivo* microscopy enables the possibility of real-time live cell imaging. Here we report a robust non-invasive method to achieve repeated and precise access to the same micro-locations over a two weeks observation window.

Methods
The technique is based on affixing a temporary skin “surface marker” as a landmark to help locating the same microstructures between imaging sessions. At baseline, the region-of-interest (ROI) is determined and imaged; at follow up sessions, the ROI can be readily revisited using the external marker. Using this method, we were able to monitor the same cells in human skin after ultraviolet B (UVB) radiation over two weeks. Skin microscopic responses were studied with a multimodality *in vivo* microscopy system.

Results
Quantitative analysis of TPF signal revealed that the melanin distribution pattern changed with time after UVB exposure with melanin appearing to migrate upwards towards the skin surface. Blood flow was monitored within the same capillaries over two weeks. Multimodal analyses enabled accurate thickness calculation of the viable epidermis, and stratum corneum as well as cell density variations over time, thus demonstrating evolution of tissue edema and cell proliferation induced by UVB.

> OC013. Oral Communication
Symposium MED-7 Imaging (Harvey Lui)

AUTOMATED IMMUNO-HISTO-ENZYMATIC INVESTIGATION OF METABOLIC ENZYME ACTIVITY AND SPATIAL ALLOCATION OF UVB-PRETREATED NORMAL HUMAN KERATINOCYTES IN EPIDERMAL SKIN EQUIVALENTS

Authors: Christopher Kremslehner[1,5], Anne Miller[2], Robert Nica[3], Francesca Ferrara[4], Michael Mildner[1], Arvand Haschemi[2], Florian Gruber[1,5]
Presenting Author: Florian Gruber
1) Medical University of Vienna, Austria, Dept of Dermatology 2) Medical University of Vienna,Austria, KIMCL 3) TissueGnostics, Vienna 4) Plants for Human Health Institute, University of North Carolina, Kannapolis, USA 5) Christian Doppler Laboratory for the Biotechnology of Skin Aging

We here report the development of an automated microscopy method that allows relating the individual enzymatic activity of single cells to immunohistochemical marker expression and its position within the epidermis of the human skin or a 3D skin equivalent (SE) model.

We adapted the StrataQuest software (TissueGnostics) to automatically pedict the epidermis based on nuclear density-mapping and to further to allow distance-based distinction between the basal and low suprabasal epidermal strata, as well as the stratum corneum on automated microscopy scans of skin sections. To validate strata prediction the sections were immunofluorescence counter-stained for differentiation markers (KRT10, KRT14). A tetrazolium-based enzymatic activity assay for G6PD was established that allows relating the native enzymatic activity to the chromogenic signal in cryosections. To analyze the influence of UVB irradiation on the metabolic activity and spatial allocation of keratinocytes SE were generated including 20% of pre-irradiated, labelled cells.

The measured G6PD activity in the different strata revealed a significant increase from the basal to the suprabasal reflecting the histologic confirmation of strong tetrazolium salt signal in the granular layer of the epidermis. Further UVB-irradiated cells in the suprabasal strata showed a reduced enzymatic activity compared to surrounding untreated cells. Based on the distribution of labelled cells within the different strata a decreased presence of UVB-irradiated cells within the basal and low suprabasal strata was detected which may indicate an increased clearance of UVB-irradiated cells via differentiation.

In conclusion, we are able to present an automated image analysis tool that reliably identifies the basal and suprabasal strata of the human epidermis, and can allocate both the spatial distribution as well as the enzyme-activity staining to pretreated or IF-detected cells with in the 3D microenvironment.

No conflicts of interest.

Reference

Miller A, Nagy C, Knapp B, Laengle J, Ponweiser E, Groeger M, Starkl P, Bergmann M, Wagner O, Haschemi A. Exploring Metabolic Configurations of Single Cells within Complex Tissue Microenvironments. Cell Metab. 2017 Nov 7;26(5):788-800.e6. doi: 10.1016/j.cmet.2017.08.014. PMID: 28889950.

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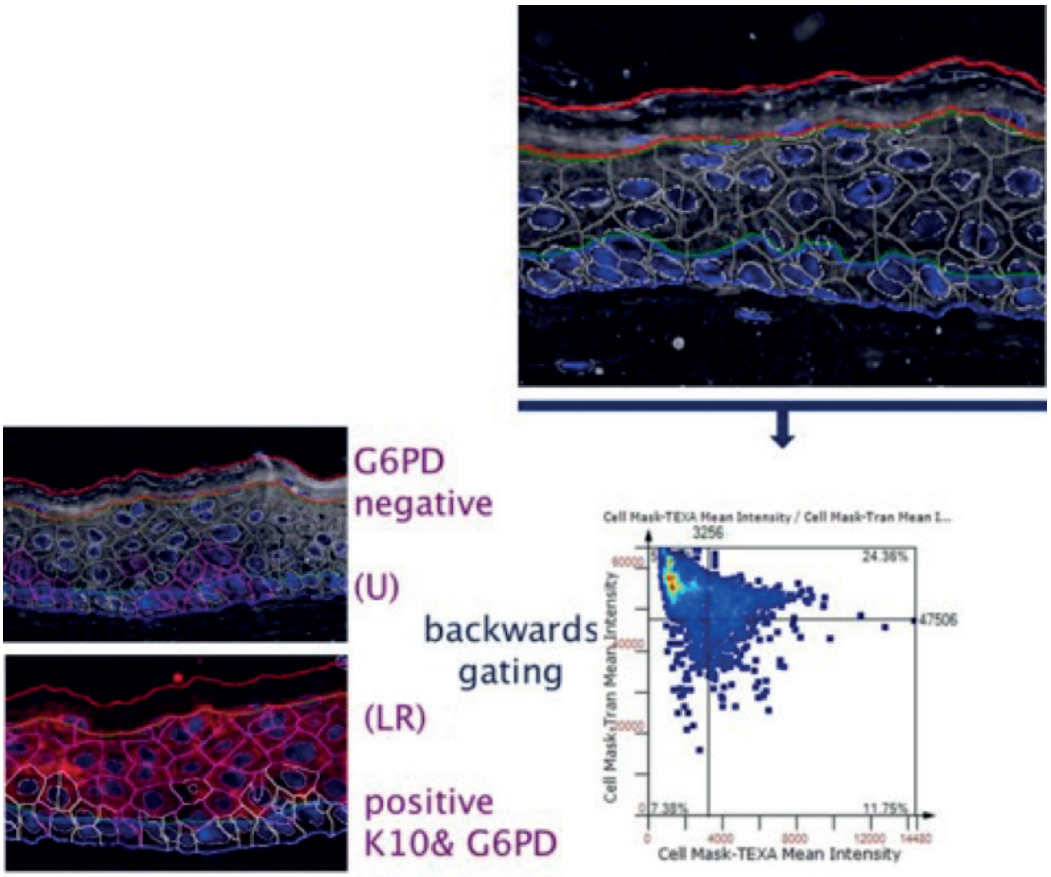
G6PD
negative
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3256
24.36%
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> P011. Poster

Symposium MED-7 Imaging (Harvey Lui)

A NOVEL FLUORESCENCE LAPAROSCOPY SYSTEM FOR INTRAOPERATIVE DIAGNOSIS AND GUIDANCE OF PDT

Authors: Soo-Jin Bae[1], Dae-Sic Lee[1], Hansuk Kim[1], Min Joo Kang[2], Keun-Ho Lee[3]
Presenting Author: Soo-Jin Bae
1) Korea Electrotechnology Research Institute 2) DongSung Bio Pharm Co.Ltd 3) Seoul St. Mary's Hospital, The Catholic University of Korea

Introduction

Pancreatic cancer is often difficult to be diagnosed early because people in early stage of pancreatic cancer have no symptom. The majority of patients are found at the inoperable advanced condition. Photodynamic therapy(PDT) has emerged as a viable treatment in inoperable patients to kill cancer cells or improve and relieve patient's symptoms. We report a novel fluorescence laparoscopy system visualizing lesion features with induced fluorescence by Photolon and autofluorescence simultaneously. This promising laparoscopic tool allows both intra-operative cancer screening and treatment monitoring during PDT.

Methods

The fluorescence laparoscopy system consists of a 10mm standard laparoscope with 30° direction and 70° field of view, an illuminator having white LED and 405nm UV LED, and an image pickup module equipped with a single color camera and an observation filter which transmit the whole spectrum range of the white LED for a conventional white light imaging mode while cut off all of reflected UV LED light for a fluorescence imaging mode. The net transmittance of the instrument in a fluorescence imaging mode allows to simultaneously detect the induced fluorescence by Photolon accumulated in tumor and the autofluorescence by endogenous fluorophores informing biological substrate condition directly and therefore, precisely identify red cancer lesions on green autofluorescence backgrounds without auxiliary background illumination. The capability of the fluorescence laparoscopy system was validated by in vivo experiments using the human pancreas tumor xenograft mouse. Photolon of 2.5mg/kg was injected into tail veins. Photolon induced fluorescence and autofluorescence were observed in normal and tumor tissues during spreading of photosensitizer and photodynamic therapy.

Results and Discussion

After administration of Photolon, we observed that red induced fluorescence diffused and accumulated higher in tumor than adjacent normal tissue, and then decreased over time. While intensity of induced fluorescence was changed over time, no observable time variation was found for the intensity of autofluorescence encompassing metabolic conditions by endogenous fluorophore. In autofluorescence imaging, tumor lesions showed cold spot compared to normal sites regardless of the concentration and diffusion of Photolon. During PDT, induced fluorescence decreased at a fast rate but autofluorescence uninfluenced.

Conclusions

We developed the laparoscopy system providing autofluorescence imaging as well as induced fluorescence imaging at the same time for intra-operative diagnosis and image-guided photodynamic therapy of pancreas cancer. Combination of induced fluorescence image and autofluorescence image could allow for enhanced discrimination between cancer and the surrounding normal tissues and monitor the progress of PDT through concentration change of Photolon.

Acknowledgements

This research was supported by the KERI primary research program of MSIT/NST (No.19-12-N0101-63)

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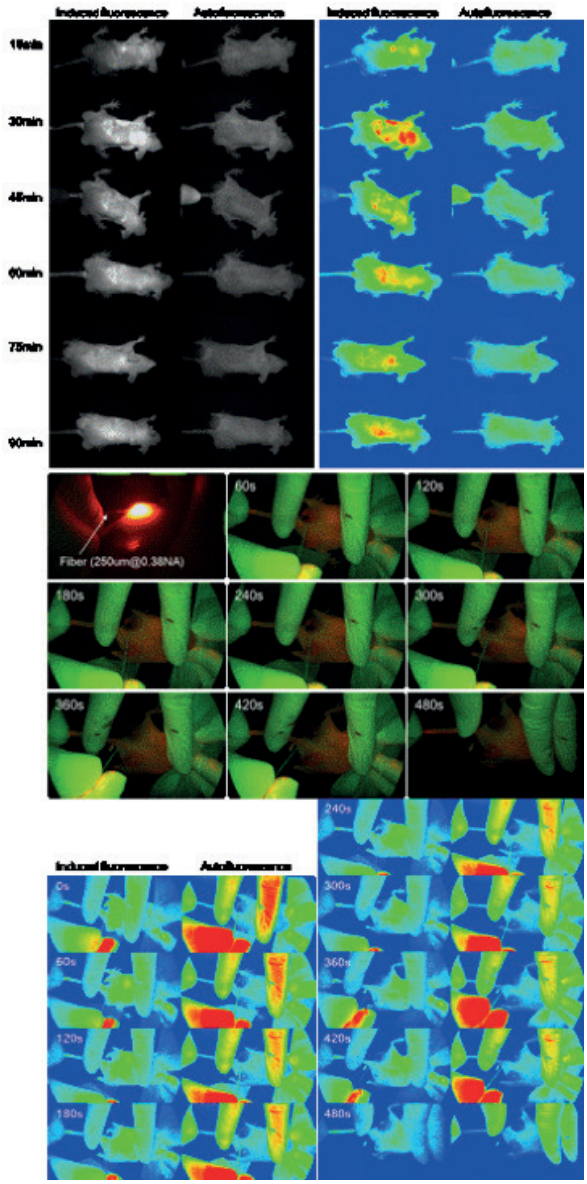
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> P012. Poster

Symposium MED-7 Imaging (Harvey Lui)

PROBING THE SURFACE OF HUMAN SKIN BIOCHEMISTRY: HOW LASERS, LIGHT SCATTERING AND MOLECULAR IONISATION IMPROVE OUR UNDERSTANDING OF DISCOID LUPUS ERYTHEMATOSUS

Authors: Hannah Holtkamp[1,2,3], Michel Nieuwoudt[1,2,3], Paul Jarrett[4], Cather Simpson[1,2,3]
Presenting Author: Hannah Holtkamp
1) School of Chemical Sciences and The Photon factory, The University of Auckland, 23 Symonds St, Auckland, NZ 2) MacDiarmid Institute for Nanotechnologies and Advanced Materials, NZ 3) Dodd Walls centre for photonics and quantum optics, NZ 4) Dermatology Department, Middlemore Hospital and Dept. of Medicine, The University of Auckland, NZ 5) Department of Physics, The University of Auckland, 23 Symonds St, Auckland, NZ

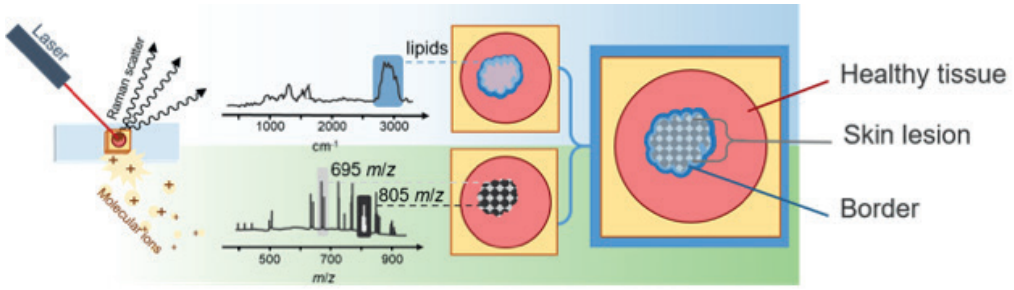
Mutimodal biomedical imaging and data fusion allows for unprecedented new insights into the biochemistry of skin disease.[1] Human skin is a chemically, spatially and temporally complex organ with biomolecules spanning concentration ranges over several orders of magnitude. To understand the biological processes occurring no single imaging technique is perfect as each has its natural limitations. Correlating two complementary techniques provides a more complete picture than either technique individually.

In New Zealand the autoimmune, photosensitive skin disease discoid lupus erythematosus (DLE) is prevalent particularly among women and Māori and Pacific Islanders, but its precise pathogenesis is uncertain. It can be difficult, particularly for non-experts, to distinguish from other skin diseases.[2] This delay in diagnosis can allow permanent facial scars to form and hair loss to result, causing significant psychological impact for patients.[3] Analysing DLE biopsies using a combination of Raman spectroscopic and mass spectrometric imaging provides a broad biochemical overview on previously unstudied biological systems, and requires no *a priori* knowledge of biomarkers. Raman spectroscopy provides a summary 'fingerprint' of the molecular skin composition of DLE while mass spectrometry identifies the individual compounds with high accuracy.

Chemometric analysis of *in vivo* Raman spectra of DLE demonstrates that DLE can be distinguished from other skin conditions by changes in its molecular composition, enabling identification of the borders between DLE lesions and healthy adjacent tissue. Co-registering Raman and mass spectrometry spectral images through data fusion techniques would enable specific biomarkers to be identified and enhance the interpretation of the Raman spectra.[4] This approach would increase the understanding of the biochemical changes instigating DLE and improve diagnostic sensitivity and specificity.

References

[1]R. Masyuko, E. J. Lanni, J. V. Sweedler, P. W. Bohn, *Analyst* **2013**, *138*, 1924-1939.
[2]P. Jarrett, S. Thornley, R. Scragg, *LUPUS* **2016**, *25*, 1497-1502.
[3] H. Gordon, A. Chandran, A. Vandal, A. Yung, P. Jarrett, *Br. J. Dermatol.* **2017**, *177*, 1134-1135.
[4]T. W. Bocklitz, A. C. Crecelius, C. Matthäus, N. Tarcea, F. von Eggeling, M. Schmitt, U. S. Schubert, J. Popp, *Anal. Chem.* **2013**, *85*, 10829-10834.



> IL047. Invited Lecture
Symposium MED-8 Phototherapy (Giovanni Leone)

NEW TECHNOLOGIES IN PHOTOTHERAPY

Authors: Giovanni Leone[1]
Presenting Author: Giovanni Leone
1) Photodermatology Unit, San Gallicano Dermatological Institute, Roma, Italy

Recent developments (new wavelengths, treatment concepts, and combinations) in the field of lasers, intense pulsed light (IPL), LED, as well as new energy and light sources have opened up new therapeutic options. Thus, while excimer lasers have now become important tools in the treatment of vitiligo and psoriasis and other dermatological diseases, the introduction of new sources with similar wavelengths like the Titanium-Sapphire Laser 311 nm. may offer new characteristics in terms of efficiency and reduction of costs. The requirements posed to physicians, both with respect to establishing the indication and conducting treatment, have been growing along with the increase in technological complexity. At the same time, LED sources emitting blue light have proved to be useful for treating psoriasis without exposing the skin to UV. These devices are characterized by low power and special safety features aimed at preventing accidents, risks, and side effects. These new technologies are reviewed and discussed in the optics of a possible use to replace, in some cases, “pure” ultraviolet phototherapy In the aforementioned setting, it is important that all potential users of these new technologies be properly trained in a manner that ensures those treated a maximum of safety and efficacy.

> IL048. Invited Lecture
Symposium MED-8 Phototherapy (Giovanni Leone)

THE PLACE OF UVA1 PHOTOTHERAPY IN DERMATOLOGY

Authors: Sally Ibbotson[1]
Presenting Author: Sally Ibbotson
1) University of Dundee

UVA1 phototherapy describes the controlled repeated use of the longer wavelengths of UVA (340-400 nm) for therapeutic purposes. It is available in specialist dermatology centres that have particular expertise in light-based therapies and may be effective in a range of diverse diseases. Whilst the introduction of UVA1 phototherapy was particularly with atopic eczema in mind, narrowband UVB and PUVA are also effective for eczema and are more widely available. UVA1 is thus of particular interest for use in conditions for which there are no other widely available effective treatments. This includes the fibrosing skin diseases, in particular scleroderma, for which there is good evidence to support the use of UVA1 for therapeutic improvement of scleroderma. Interestingly, some of the most robust evidence for UVA1 phototherapy has been shown in randomised controlled trials using very low doses in systemic lupus erythematosus, with improvement shown in both systemic and cutaneous disease. UVA1 phototherapy is generally well tolerated, with minimal adverse effects. However, UVA1 can induce DNA damage in the basal layer and upper dermis and thus there is photocarcinogenic potential, which has not been shown with human use but this needs to be kept under observation. At present, UVA1 phototherapy should be available in tertiary dermatology centres, and for some patients can be an invaluable treatment option. Further evaluation of its use and place as a treatment option for a variety of diseases needs to be confirmed through further robust clinical trials.

> IL049. Invited Lecture
Symposium MED-8 Phototherapy (Giovanni Leone)

PHOTOCHEMOTHERAPY OF CHRONIC ACTINIC DERMATITIS

Authors: Ljubomir Novakovic[1,2]
Presenting Author: Ljubomir Novakovic
1) Queen Elizabeth Hospital, Greenwich 2) St. John's Institute of Dermatology, Guy's Hospital, London

Chronic actinic dermatitis (CAD) is a debilitating and often recalcitrant photodermatosis which significantly impacts on quality of life. Diagnosis is based on clinical evidence of eczematous skin changes on exposed sites and abnormal phototest responses, i.e. reduced minimal erythema dose with solar simulator and abnormal sensitivity on monochromator testing.

First line therapy consists of strict photoprotection and treatment of dermatitis with topical or calcineurin inhibitors. Patients with severe disease may require second line therapies such as systemic immunosuppressants. PUVA is another option which may be useful in patients who are unwilling or unable to commence systemic treatment. However, there are to date very few case reports of PUVA being used in CAD. It is possible that PUVA has been overlooked as a treatment option due to concerns of UVA flaring up CAD.

A recent six year retrospective study conducted at St. John's Institute of Dermatology in London looked into sixteen cases of treatment-resistant CAD.[1] It confirmed that oral PUVA, started at the very low initial doses of UVA and with very small UVA dose increments, can be effective in over 60% of patients with CAD. PUVA causes fewer flares of CAD than expected. Although this is the largest case series to date in the literature, the patient population was too small to draw conclusions regarding factors which may predict the efficacy of PUVA.

1 Chee SN, Novakovic L, Fassihi H, Garibaldinos T, Sarkany R. Chronic actinic dermatitis: successful treatment with psoralen-ultraviolet A photochemotherapy. *Br J Dermatol* 2018; 178(3): e189-e190.

> IL050. Invited Lecture
Symposium MED-8 Phototherapy (Giovanni Leone)

PHOTOTHERAPY OF VITILIGO

Authors: Agustín Alomar[1]
Presenting Author: Agustín Alomar
1) Hospital Dexeus

In my opinion it is very important to accept that all treatments need light and therefore the difference between healthy and affected skin will be more visible.

To obtain success in vitiligo treatment the combination with light seems totally necessary.

I will review several forms of phototherapy:
PUVA therapy, UVB-NB, Lesion target phototherapy - 308 excimer light, UVB devices.

Some results in relation to research about the stimulation and progression of repigmentation will be presented.

And finally, again in my modest opinion, combination therapies always plus light is the best possibility to obtain the best responses

> IL051. Invited Lecture
Symposium MED-8 Phototherapy (Giovanni Leone)

PHOTOTHERAPY VERSUS NEW DRUGS FOR ATOPIC DERMATITIS

Authors: Piergiacomo Calzavara-Pinton[1]
Presenting Author: Piergiacomo Calzavara-Pinton
1) University of Brescia

Untl 2 years ago, high dosage (5mg/kg bw) cyclosporine was the only drug that was approved by EMA for severe atopic dermatitis. It was very successful for the short term control of the disease but its chronic use was limited by the risk of serious adverse. UVA, UVAB, UVA1, UVB-BB, UVB-NB and PUVa were used widely worldwide to control acute flares, but again they were not useful for the long term control of the disease.

A growing number of anti IL4, IL 13 and IL 31 biologics and new anti JAK1 and JAK2 topical and systemic drugs are rapidly changing the therapeutic scenario. However thse drugs does not substitute phototherapies because they can still be useful for the control of the flares occurring under treatment with new drugs as well in cases with partial improvement,

> IL052. Invited Lecture
Symposium MED-8 Phototherapy (Giovanni Leone)

PHOTOTHERAPY FOR PSORIASIS IN THE ERA OF BIOLOGICS AND SMALL MOLECULE INHIBITORS

Authors: Henry W. Lim[1]
Presenting Author: Henry W. Lim
1) Department of Dermatology, Henry Ford Hospital, Detroit, Michigan, USA.

Phototherapy is a long standing treatment modality in dermatology. Currently, this include narrowband (NB)-UVB, targeted phototherapy, UVA1 (340-400 nm) and psoralen plus UVA (PUVA) photochemotherapy. UVA1 is used primarily for sclerodermoid disorder such as morphea, scleredema, and progressive systemic sclerosis. NB-UVB, targeted phototherapy and PUVA are all established and cost- effective treatment modalities for psoriasis, as well as many other dermatoses (atopic dermatitis, vitiligo, some photodermatoses). Based on currently available data, NB-UVB is not associated with increased risk of skin cancer. However, photocarcinogenesis secondary to PUVA has been well-reported. The long term side effects of targeted phototherapy and UVA1 are not known; however, since both are administered for relatively short duration, they are most likely as safe.

Biologics and small molecule inhibitors are important and exciting advancement in the treatment of psoriasis. Some can achieve almost complete clearance of psoriasis. The earliest approval in the United States for this class of medications was in 2004 (etarnecept); while they are safe when used for appropriate patients and with careful monitoring, risk of immusuppression needs to be carefully considered. Furthermore, as these are extremely expansive medications, proper patient selection, including prior therapy, is essential.

> OC014. Oral Communication
Symposium MED-8 Phototherapy (Giovanni Leone)

UVA THERAPY & UVA ENHANCES BRUSATOL-MEDIATED INHIBITION OF MELANOMA GROWTH

Authors: Mei Wang[1], Julia Li Zhong[1]
Presenting Author: Julia Li Zhong
1) College of Bioengineering & Dermatology Unit, University Hospital, Chongqing University, China

Introduction
UVA therapy is broadly used for skin conditions, such as vitiligo, eczema and psoriasis etc. UVA irradiation generates ROS can damage both normal and cancer cells and may be of potential use in phototherapy. Brusatol (BR) is a potent inhibitor of Nrf2, a transcription factor that is highly expressed in cancer tissues and confers chemo-resistance. In order to provide an alternative method to treat the aggressive melanoma, we sought to investigate whether low-dose UVA with BR is more effective in eliminating melanoma cells than the respective single treatments.

Methods
Cell viability was measured by MTS Assay, Western Blot, Immunofluorescence and QPCR was used to analyze gene expression.

Results and Discussion
We found that BR combined with UVA led to inhibition of A375 melanoma cell proliferation by cell cycle arrest in the G1 phase and triggers cell apoptosis. Furthermore, inhibition of Nrf2 expression attenuated colony formation and tumor development from A375 cells in heterotopic mouse models. In addition, co-treatment of UVA and BR partially suppressed Nrf2 and its downstream target genes such as HO-1 along with the PI3K/AKT pathway.

Conclusions
We propose that co-treatment increased ROS-induced cell cycle arrest and cellular apoptosis and inhibits melanoma growth by regulating the AKT-Nrf2 pathway in A375 cells which offers a possible therapeutic intervention strategy for the treatment of human melanoma.

Acknowledgements
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Conflicts of Interest
The authors declare no competing interests.

References

Mounessa J, Buntinx-Krieg T, Qin R, et al. Primary and Secondary Chemoprevention of Malignant Melanoma[J]. American Journal of Clinical Dermatology, 2016, 17(6):1-10.

Constantinou M. Melanoma Genomics and Immunotherapy.[J]. Rhode Island Medical Journal, 2015, 98(11):31.

> OC015. Oral Communication
Symposium MED-8 Phototherapy (Giovanni Leone)

ACTION SPECTRUM OF NITRIC OXIDE RELEASE FROM SKIN

Authors: Bethany Ferris[1], Richard Weller[1]
Presenting Author: Bethany Ferris
1) Queen's Medical Research Institute, University of Edinburgh

Introduction

Hypertension accounts for 9.4 million annual global deaths from vascular disease. Nitric oxide (NO) is a vasodilator which can be photolysed from nitrate (NO3), nitrite (NO2) and other nitroso-compounds, such as thiols, in the skin. Ultraviolet (UV) light has been shown to generate NO in the epidermis. Furthermore, whole-body irradiation with UV-A light reduces blood pressure in healthy volunteers. The specific wavelength responsible for NO release from skin is unknown. Recent research has shown that keratinocytes exposed to UV-A show a marked dose-dependent increase in NO, greater than that for UV-B however, other cells in the skin may also be responsible for NO release. This research looks at the irradiation of whole human skin specimens using narrow wavelengths of light.

Method

Skin donated from elective plastic surgery operations was cut using a 6mm punch biopsy tool. Each specimen was irradiated with broadband UV light, from a xenon arc monochromator, and chemiluminescence detection used to measure the concentration of NO released. Different filters were used to alter the wavelengths of light (FWG-32050, FGG-40050, 300FS10-50, CDC-5051) and an action spectrum was calculated for each filter. To minimise bias caused by inter-individual variation in skin nitroso-compounds, each skin specimen was analysed using each filter.

Results

Skin irradiated with UV-A light showed the most marked dose-dependent increase in NO followed by visible and blue light. Irradiation with UV-B did not elicit a dose-dependent increase in NO. Repeated irradiation with UV and visible light did decrease the magnitude of NO release from the skin.

Conclusion

This ongoing research suggests that UV-A is more important than UV-B for the photolysis of nitroso-species in human skin. Further work will look at irradiating skin with specific wavelengths of light in the UV-A and visible spectrum.

Acknowledgements

Spire Hospital, Edinburgh

Conflicts of Interest

None to declare

References

Moncada, S., Palmer, R.M.J. & Higgs, E.A. The Discovery of Nitric Oxide as the Endogenous Nitrovasodilator. Hypertension 12, 365-372 (1988).

Weller, R.B. Sunlight Has Cardiovascular Benefits Independently of Vitamin D. Blood Purification 41, 130-134 (2016).

Liu, D., Fernandez, B.O., Hamilton, A. et al. UVA Irradiation of Human Skin Vasodilates Arterial Vasculature and Lowers Blood Pressure Independently of Nitric Oxide Synthase. J Invest. Dermatol. 134, 1839-1846 (2014).

Oplander, C., Volkmar, C.M., Paunel-Görgülü, A. et al. Whole Body UVA Irradiation Lowers Systemic Blood Pressure by Release of Nitric Oxide From Intracutaneous Photolabile Nitric Oxide Derivates. Circ. Res. 105, 1031-1215 (2009).

Mowbray, M., McLintock, S., Weerakoon, R. et al. Enzyme-Independent NO Stores in Human Skin: Quantification and Influence of UV Radiation. J Invest. Dermatol. 129, 834-842 (2009).

Holliman, G., Lowe, D., Cohen, H. et al. Ultraviolet Radiation-Induced Production of Nitric Oxide: A multi-cell and multi-donor analysis. Scientific Reports 7 (2017).

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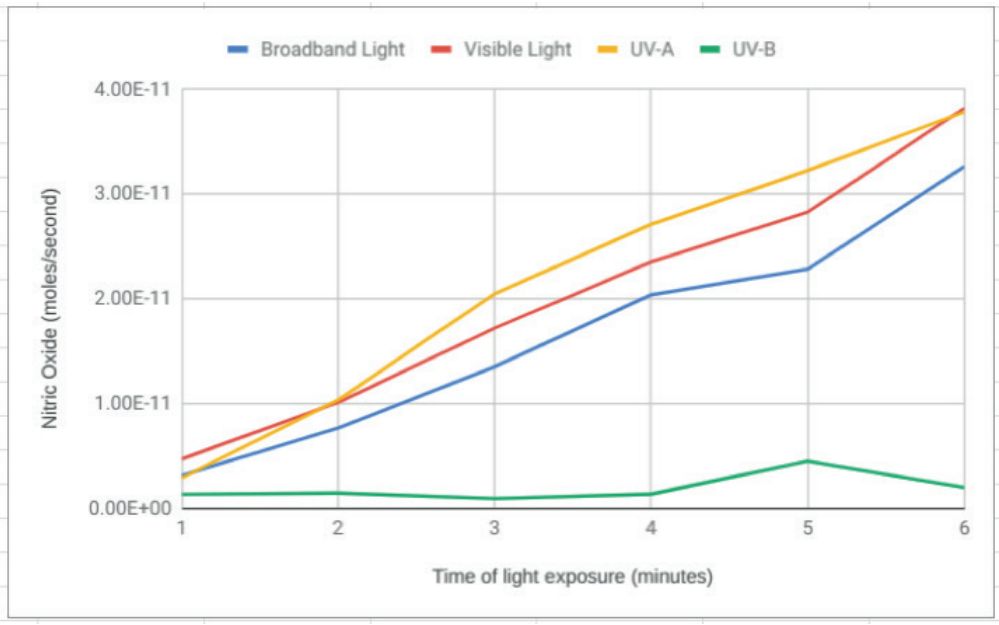
Broadband Light
Visible Light
UV-A
UV-B
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3.00E-11
2.00E-11
1.00E-11
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Nitric Oxide (moles/second)
1
2
3
4
5
6
Time of light exposure (minutes)

> P013. Poster

Symposium MED-8 Phototherapy (Giovanni Leone)

DEVELOPMENT AND PRELIMINARY INVESTIGATIONS OF A WEARABLE DEVICE FOR HIGH RESOLUTION TEMPORAL MEASUREMENTS OF ERYTHEMA

Authors: Paul O'Mahoney[1 2 3], Alex Thompson[4], Saydulla Persheyev[4], Ewan Eadie[2 3], Sally Ibbotson[1 2 3], Ifor Samuel[4], James Ferguson[2]
Presenting Author: Paul O'Mahoney
1) School of Medicine, University of Dundee, Dundee, UK 2) Photobiology Unit, NHS Tayside, Dundee, UK 3) Scottish Photodynamic Therapy Centre, Dundee, UK 4) School of Physics and Astronomy, University of St. Andrews, St. Andrews, UK

Erythema is reddening of the skin in response to an insult such ultraviolet (UV) radiation. By controlled exposure with UV radiation, a minimal erythema dose (MED) indicative of threshold erythemal sensitivity may be determined. The MED of a specific patient on a specific skin site is an important measurement – it can allow for determination of initial dose in UV phototherapy, provides an indication of photosensitivity in diagnostic phototesting and may be influenced by factors, such as photoactive drugs.

Due to increased blood flow at the UV-exposed site presenting reddening of the skin, the spectral reflectivity of skin is altered, and this change allows for measurement of the erythema index (EI) using reflectivity techniques, which involves shining red, green and far-red light on to the skin and measuring the reflected light on a photodetector. While the ratio between reflected red and green wavebands of light gives an indication of EI, the ratio of red and far-red gives the pigmentation index (PI). In this manner, EI can be determined irrespective of baseline skin pigmentation. Current commercial instrumentation for determining EI provide data at a single time point, with repeated measurements required to build a time series. The subject must have repeated visits for subsequent measurements, making detailed time course erythema measurements impractical and leaving gaps in our understanding of the characteristics of UV-induced erythema.

A wearable device for real-time monitoring of induced erythema has been designed, fabricated and tested in a collaboration between the Photobiology Unit at NHS Tayside and the University of St. Andrews. The device may be worn, and measurements of erythema recorded at set intervals (approximately every 30 seconds) for up to 48 hours, enabling continuous assessment of the development and time-course of UV-induced erythema.

We present the development of the device and preliminary data relating to early use in humans. This facilitates in detail, practical analysis of induced erythema over a prolonged time period and furthers our understanding of the characteristics of the erythemal response to irradiation.

> P014. Poster

Symposium MED-8 Phototherapy (Giovanni Leone)

NARROW-BAND UVB TREATMENT OF VITILIGO IN CHILDREN

Authors: Hanna Rinner[1], Peter Wolf[1], Alexandra Gruber-Wackernagel[1], Franz J. Legat[1], Angelika Hofer[1]
Presenting Author: Angelika Hofer
1) Medical University of Graz, Department of Dermatology and Venereology

Introduction

Vitiligo is an acquired skin disorder characterised by depigmented patches of the skin. Approximately half of all cases develop first skin lesions in childhood or adolescence. With limited data available concerning phototherapy of vitiligo in young patients, the aim of this retrospective case study was to analyse the efficacy of UVB 311nm phototherapy in infants and adolescents.

Patients/Methods

The analysis includes data from 52 vitiligo patients younger than 19 years who had been treated with UVB 311nm phototherapy between January 2003 and February 2018. Thirty-one young patients were treated at the Photodermatology Unit, Department of Dermatology, Medical University of Graz, Austria and twenty-one patients underwent phototherapy at private dermatology offices. Narrow-band UVB was given twice a week for 3 months. Only in case of good repigmentation (>50%) the administration of treatment had been extended beyond 3 months. Age of onset, gender, vitiligo classification, co-morbidities, family history,phototherapy characteristics, including cumulative dose, number of radiation treatments and duration of the treatment as well as the administration of additional topical agents were analysed. The data were retrieved from the electronic health and patient record database of our department or patient reports of private dermatology offices. Phototherapy induced repigmentation was assessed by comparing photos taken at our department before and after therapy. The study was approved by the Ethics Committee of the Medical University of Graz.

Results

Seventy-five percent (75%) (39 out of 52 pts) treated with UVB 311nm therapy achieved repigmentation. The mean treatment number was 36 (range, 8 to 87). The mean cumulative dose was 28,2 J/cm^2 (range, 6,5 to 81,9). Seventeen patients (33%) achieved repigmentation greater than 50%. In five patients we saw further progression of vitiligo during phototherapy. Two of them were diagnosed with autoimmune thyroiditis. Slight erythema necessitated a transient dose reduction in 7 out of 52 patients.

Conclusion

Our data show that narrow-band UVB phototherapy is an effective treatment modality in children and adolescents with vitiligo.

> P015. Poster

Symposium MED-8 Phototherapy (Giovanni Leone)

TARGETED DIGITAL PHOTOTHERAPY AS TREATMENT FOR VITILIGO

Authors: Thomas Graier[1], Angelika Hofer[1], Peter Wolf[1]
Presenting Author: Angelika Hofer
1) Medical University of Graz, Department of Dermatology and Venereology

Introduction

Phototherapy with UVB light represents an important therapeutic option in the treatment of vitiligo. When phototherapy is administered to these patients in a non-targeted fashion, UV-induced tanning of healthy skin is a non-desired side effect. Indeed, an increased difference in skin color between healthy and diseased skin is a disadvantage with regard to cosmetic outcome. Moreover, exposure of healthy skin during phototherapy may increase the risk of skin cancer and premature skin aging. Targeted phototherapy aims to avoid exposure of healthy skin. The skintrek equipment (Lüllau Engineering, Adendorf, Germany) with its integrated camera, exposure head (with microprocessor devices, producing pixels rays by 0,27 x 0,27 mm), and computer software allows automatic and precise detection and UV exposure of diseased skin. Importantly, UV dosage can gradually be decreased by software control around the edges of skin lesions, minimizing the risk of rim-like hyperpigmentation. The use of skintrek PT3 has recently been described for the treatment of patients with psoriasis and mycosis fungoides (1,2).

Methods

This represents a retrospective case series of two patients with generalized vitiligo and two patients with localized vitiligo who had received targeted UVB phototherapy using skintrek PT3 for depigmented facial lesions, persisting for 16 months on average. Expansion of lesions was compared prior and after treatment, as well as overall pigmentation in the areas affected.

Results

Patients received phototherapy twice a week for 10 weeks followed by treatment once a week for 4 weeks (cumulative UV doses ranged from 11,36 to 15,7J/cm^2). Progression of lesions could not be observed, neither did any new lesions occur during therapy. In fact, repigmentation was found in all lesions of 3 patients with reduction in depigmented areas of >60%, >50% and >20%, respectively. One patient with localized vitiligo showed no response to digital phototherapy. No tanning was observed in lesion-adjacent skin.

Conclusion

This study provides proof-of-principle for digital phototherapy as a new therapeutic approach for phototherapy in patients suffering from vitiligo. The fact that only diseased skin is exposed but healthy skin is entirely spared during digital phototherapy makes this approach very attractive and most likely more safe than conventional phototherapy in the treatment of vitiligo.

References

1. Werfel T et al. Digital ultraviolet therapy: a novel therapeutic approach for the targeted treatment of psoriasis vulgaris. Br J Dermatol. 2015;172(3):746–53.
2. Reidel U et al. Treatment of localized mycosis fungoides with digital UV photochemotherapy. Photodermatol Photoimmunol Photomed. 2015;31(6):333–40.

> P016. Poster

Symposium MED-8 Phototherapy (Giovanni Leone)

SPECTRALLY AND SPATIALLY RESOLVED DEPTH PENETRATION ACHIEVED BY PHOTOTHERAPY LAMPS

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Introduction

The majority of ultraviolet phototherapy is performed using narrowband ultraviolet-B (NB-UVB) radiation. However there remains an important role for both PUVA (Psoralen + ultraviolet-A) photochemotherapy and, less commonly, UVAI phototherapy. The radiation sources used in phototherapy treatment are broadband fluorescent or metal-halide lamps (1). As the penetration depth achieved by radiation incident on the skin is wavelength dependent, we investigate the skin penetration depths of different phototherapy radiation sources.

Methods

Monte Carlo radiative transfer (MCRT) methods use localised scattering and absorption probabilities to describe the path of photon packets through a medium. MCRT methods are ideally suited to modelling a complex structure such as the upper layers of the skin (2), as multiple physical quantities can be measured with spatial resolution limited only by computational power available.
Using a modified version of a previously published multilayered MCRT skin model (3), irradiation by several phototherapy light sources are simulated. The wavelength dependent fluence at depth achieved by each light source is recovered. In addition, the skin model as published was altered to better simulate psoriatic tissue.

Results & Discussion

We find that UVA I (both fluorescent and metal halide lamps) provide a depth penetration advantage over that achieved by broadband UVA and narrowband UVB. The depth at which 10% of incident radiation remains is 40μm deeper for UVA I than for a UVA source; and 130 μm deeper than for a NB-UVB source.
We present the spectra recovered at depth for different phototherapy lamps, and the spectra incident on the basal layer and the dermis. In addition, we present results indicating depth penetration of different lamp sources in simulated psoriatic skin treated with Psoralen (4).

Conclusions

In conclusion, our results provide depth of penetration values for the common UV phototherapy sources. As the emission spectrum of UVA I sources crosses over the absorption spectrum of psoralen, UVA I radiation may be suitable for PUVA treatment in cases where deeper depth penetration is required.

Acknowledgements

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Conflicts of Interest

None

References

1. Hönigsmann, H. (2001). Phototherapy for psoriasis. *Clinical and experimental dermatology*, *26*(4)
2. Wang, L. et al (1995). MCML—Monte Carlo modeling of light transport in multi-layered tissues. *Computer methods and programs in biomedicine*, *47*(2)
3. Barnard, I. R. M. et al (2018). Quantifying direct DNA damage in the basal layer of skin exposed to UV radiation from sunbeds. *Photochemistry and photobiology*, *94*(5)
4. Yeargers, E. et al (1965). Absorption and emission spectra of psoralen and 8-Methoxypsoralen in powders and in solutions. *Journal of Investigative Dermatology*, *44*(3)

> P017. Poster

Symposium MED-8 Phototherapy (Giovanni Leone)

PHOTODAMAGE OF CELLS SENSITIZED WITH BILIRUBIN UPON EXPOSURE TO LASER AND LED SOURCES

Authors: Ihar Leusenka[1], Tatsiana Ananich[1], Ludmila Plavskaya[1], Vasili Katarkevich[1], Aliaksandr Mikulich[1], Antonina Tretyakova[1], Andrey Sobchuk[1], Vitaly Plavsky[1]
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Over the past years, a greater concern has emerged about possible side effects of phototherapy of neonatal hyperbilirubinemia dealing with sensitizing effect of bilirubin and its photoproducts. The data that application of phototherapy in infants with extremely low birth weight (500 – 700 g) may adversely affect their health status exacerbated interest to this problem. The relevance of discussed problem has become even more acute due to use for therapeutic purposes of new radiation sources (super-bright LEDs) that allow varying in a wide range both the intensity and the wavelength of acting radiation within absorption band of bilirubin. At the same time, the appearance on the market of semiconductor lasers emitting in blue spectral region raised the question about prospects for using laser sources in phototherapeutic equipment.

The aim of this work is to study the regularities of bilirubin-sensitized photodamage of BGM cells under exposure to laser and LED sources.

It is shown that bilirubin and its photoproducts localized in cell compartments are capable of causing photosensitized death of cells. A characteristic feature of dose-response curves for cell viability is their mono-exponential character. This indicates a constant rate of photodamage of cells during irradiation. Another distinctive feature of dose-response curves is practically identical photobiological effect upon exposure to radiation of the LED source with λ_{max} = 465 nm, corresponding to the maximum absorption spectrum of bilirubin in complex with albumin, and the radiation with λ_{max} = 520 nm, corresponding to the long-wavelength slope of this spectrum. In our opinion, this indicates either the participation of bilirubin photoproducts in the effect of sensitization or the heterogeneous character of distribution and localization of bilirubin in cells. In this case, bilirubin, bound with various cellular structures, is characterized by different spectral characteristics.

A pronounced dependence of cellular viability in presence of bilirubin on the wavelength of the laser irradiation upon its variation in the range of the bilirubin absorption spectrum 457.9 - 514.5 nm (irradiation time t = 5 min, power density 10 mW/cm^2) was revealed. It is shown that the greatest damaging effect is observed upon exposure of cell monolayer to radiation with λ = 514.5 nm or 457.9 nm. Exposure to radiation with wavelengths of 476.5 nm, 488.0 nm and 496.5 nm weakly influences the viability of cells.

It is shown that at close wavelengths of monochromatic laser radiation (457.9 or 514.5 nm) and radiation from LED source (465 or 512 nm) the phototoxic effect on cells pre-incubated with bilirubin is much more pronounced when cells exposed to non-monochromatic radiation.

It has been established that the main intermediate of photodamage of cells upon their sensitization with bilirubin is singlet oxygen, since addition of sodium azide to the medium with cells before irradiation practically blocks cell death.

> P018. Poster

Symposium MED-8 Phototherapy (Giovanni Leone)

LIGHT PROPAGATION THROUGH COLLOID-POLYMER MIXTURES: TOWARDS UNIFORM IRRADIANCE SOURCES FOR PHOTOTHERAPY APPLICATIONS

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Introduction

Emission uniformity of light sources is a very desirable property in phototherapy. This is true in dermatology, where non-uniform illumination of the selected skin area(s) can be associated with insufficient radiation in some points and dangerously excessive in others. Another case is represented by *in vitro* experiments to measure e.g. PDT efficacy in the case of new photosensitizers and/or photosensitizer carriers, where a constant radiant exposure at the sample level (W/cm^2) is needed for a uniform sample treatment.

Methods

To obtain a uniform light emission, we developed and characterized a diffusing gel in the visible-UVA-UVB bands, made out of two biocompatible materials: Intralipid® and methocel. Intralipid® is composed of soy fat droplets and egg yolk phospholipids suspended in water, used for parenteral nutrition; due to its diffusion properties, it is used as a light scattering medium for tissue phantom studies. Methocel is a cellulose-derived polymer, with a good transparency across the whole UV-visible spectral range and stable over time. By dispersing Intralipid® into methogel we have obtained a diffusing, non liquid and easily handable material, to enable future definition of a gel-like illuminator, whose light-diffusion properties are exploited to obtain a uniform illumination source for phototherapy.

Light propagation through the diffusing gel has been studied by varying both the Intralipid® concentration and the material thickness. To this aim, light has been injected in the mixture by external UVA illumination (LED sources), undergoing scattering mainly by the Intralipid® component. To quantify light emission (e.g. radiant exposure), we used a Gafchromic® EBT3 film dosimeter [3], whose 2D darkening response was analysed by film scanning and subsequent image analysis methods to obtain radiant exposure maps of the light emitted by the mixture and received by an illuminated surface. The illumination profiles were correlated with the injected light parameters, mixture thickness and Intralipid® concentration.

Results and Discussion

The obtained results show that emission uniformity increases at both increasing thickness and concentration, accompanied on the other side by a decrease in radiant exposure.

Conclusions

External LED light injection into a biocompatible and diffusing gel is a promising and inexpensive way towards the use of uniformly-emitting sources in phototherapy.

Acknowledgements

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References

[1] Papageorgiou P. et al (2000) *British journal of Dermatology*, *142*(5), 973-978.
[2] Nicolaidou E., et al (2009) *Journal of the American Academy of Dermatology*, *60*(3), 470-477.
[3] Borca V. C., et al (2013) *Journal of applied clinical medical physics*, *14*(2), 158-171.

> IL059. Invited Lecture
Symposium MED-9 Photosensitivity diseases (Sally Ibbotson)

WHAT'S NEW ON POLYMORPHIC LIGHT ERUPTION

Authors: Peter Wolf[1]
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Much advances have been made in recent years in understanding the pathophysiology of polymorphic light eruption (PLE), the most common form of photodermatoses. In particular, the interplay between the immune system (with UV resistance of Langerhans cells and impairment of neutrophil skin infiltration), the defense through antimicrobial peptides combined with an inadequate suppression of adaptive immunity, and putative photoallergens from UV-modified proteins released from apoptotic cellsand/or triggers produced by the skin's microbiota seem to be involved in pathophysiology of the disease. A variety of cytokines, including the itch cytokine IL-31 may be crucial players in the formation of the skin rash of the disease. These findings open avenues for the development of novel treatment strategies in PLE, including the administration of certain biologics targeting cytokines such as IL-31.

> IL055. Invited Lecture
Symposium MED-9 Photosensitivity diseases (Sally Ibbotson)

SOLAR URTICARIA

Authors: Christophe Bedane[1]
Presenting Author: Christophe Bedane
1) CHU LIMOGES

Solar urticaria(SU) is a rare type of physical urticaria triggered by sun exposure. Mechanism of action is a type 1 hypersensitivity, Immunoglobulin E mediated, triggered by an unknown photoallergen. The first line treatment is antihistamines treatment and sun avoidance. The objective of this study was to investigate the variation in results phototests in patients with solar urticarial resistant to antihistamines receiving two injections per month omalizumab 150 mg for three months. A single-center prospective study concerning four Patients with SU resistant to antihistamine treatment was carried out. The UVA, UVB and visible light phototestswith determination of the minimal urticaria dose (MUD) were performed before and after 3 months of treatment with omalizumab 150 mg twice a month, depending on the spectrum of action responsible for the appearance of lesions for each patients. Improvement of phototests and clinical signs was recorded for all patients. Omalizumab is a monoclonal antibody that binds selectively to human immunoglobulin E. Originally used in asthma; this treatment has today the authorization for use in chronic urticaria. Four other cases published with solar urticaria were successfully treated with omalizumab, another case had a partial improvement and another described a failure of with treatment. Recently two other cases of SU improved with this treatment. A multicentry phase 2 study suggests that omalizumab is an interesting therapeutic option in refractory solar urticaria despite a response to insufficient primary endpoint. Omalizumab may represent an option if antihistamines fail in solar urticarial with few side effects compared to other therapeutic options.

> IL057. Invited Lecture
Symposium MED-9 Photosensitivity diseases (Sally Ibbotson)

IMPORTANCE OF GENOTYPE–PHENOTYPE CORRELATION IN XERODERMA PIGMENTOSUM

Authors: Hiva Fassihi[1]
Presenting Author: Hiva Fassihi
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Xeroderma pigmentosum (XP) is a rare disorder of DNA repair, characterized by progressive pigmentary changes at exposed sites and a significantly increased risk of ultraviolet radiation (UVR)-induced skin cancers. About 50% of affected individuals are photosensitive, with an exaggerated and prolonged sunburn response on minimal exposure, and about 30% develop progressive neurological degeneration. XP is divided into eight complementation groups, XP-A through to XP-G and XP variant, corresponding to the affected DNA repair gene. The majority of patients are mutated in one of seven genes – XPA, XPB, XPC, XPD, XPE and XPG – whose products are involved in nucleotide excision repair of UVR and other types of DNA damage. However, about 20% of patients, with XP variants, have normal nucleotide excision repair but are defective in DNA polymerase eta, a specialized DNA polymerase required to replicate DNA past unrepaired UVR induced lesions.

In April 2010, the UK Xeroderma Pigmentosum Multi-disciplinary Service was established at St Thomas' Hospital in London. We now have over 100 patients visiting the clinic on a regular basis and on each visit the patients are examined by a group of specialists from different disciplines. Cellular analysis of DNA repair levels and molecular analysis to determine the causative XP mutation are part of the service. This has enabled the detailed genotype-phenotype examination of these patients.

Historically, XP was considered a single disease with similar features across the different complementation groups. However, it is now becoming evident that patients with XP are a clinically heterogeneous group with wide variability in clinical features both between and within XP complementation groups, in part explained by the precise nature of the pathogenic mutation(s). The detailed study of genotype-phenotype correlations in the UK XP population has improved our ability to provide prognostic information to these families.

> IL053. Invited Lecture
Symposium MED-9 Photosensitivity diseases (Sally Ibbotson)

QUALITY OF LIFE AND PSYCHOLOGICAL COMORBIDITY IN THE PHOTODERMATOSES

Authors: Kirsty Rutter[1], Iqra Ashraf[1], Lis Cordingley[1], Lesley Rhodes[1]
Presenting Author: Kirsty Rutter
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The photodermatoses (photosensitivity disorders affecting the skin) affect large proportions of the population, but relatively little is understood about the impact of these conditions on patients' quality of life (QoL) and psychological health, despite the substantial behavioural restrictions that are often necessary for management. Several assessment tools are widely available to evaluate QoL and psychological impact, including generic and skin-specific instruments.

We systematically reviewed available literature to identify tools that have been used to evaluate QoL and psychological impact, and used the data gained using these tools to quantify these impacts. A systematic search of Pubmed, OVID Medline, PsycInfo and CINAHL was conducted for articles published between 1960 and September 2018 that included assessment of QoL and psychological health in the photodermatoses. 20 studies were included in our review; 19 incorporated QoL assessment while 3 included evaluation of psychological morbidity. Six QoL tools were used: Dermatology Life Quality Index (DLQI), Children's DLQI, Family DLQI, Skindex (versions 16 and 29), Erythropoietic Protoporphyria Quality-of-Life (EPP-QOL) and EuroQoL. Data using the most commonly used tool, DLQI, showed that 31-39% photodermatoses patients experience a very large impact on QoL (DLQI>10). Particularly high impact was found on employment/education, social/leisure activities and clothing choices. Only one tool was specifically designed for a photodermatosis, i.e. the EPP-QOL, and this appeared more sensitive than the DLQI. Four tools were used to evaluate psychological impact: the Hospital Anxiety and Depression Scale (HADS), Fear of Negative Evaluation (FNE), brief COPE and Illness Perception Questionnaire-Revised (IPQ-R). Levels of anxiety and depression were approximately double British population data. Patients with facial involvement, female gender and younger age at onset showed higher psychological morbidity.

Thus, several tools have been used to assess QoL in the photodermatoses and substantial impact of these conditions on QoL has been seen. However, development of photosensitivity-specific QoL tools might better address their unique impacts. High psychological impact is also observed; more research studies are required to examine this, alongside measures to address the negative impact on patients.

> IL058. Invited Lecture
Symposium MED-9 Photosensitivity diseases (Sally Ibbotson)

PHOTOPROTECTION IN PHOTOSENSITIVITY DISEASES

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Introduction
Diseases provoked by sunlight are rather common, with UVB, UVA, and blue light as the wavelengths mostly involved. People's extensive travel activity may increase their received doses of UVR. People who are not affected by their disease in their home country in Northern Europe may manifest their photosensitivity disease when exposed to much higher UVR doses on vacation, e.g. in Southern Europe. Individuals may be sensitive to all parts of the ultraviolet radiation spectrum or specifically to UVB, UVA, or blue light. Traveling to sunny holiday destinations may also result in exposure of larger skin areas, as less clothing are worn.

Method
Methods of protection are well-known and have been advised to the public for many years: Use clothes and hats with a rim, stay out of the midday sun, stay in the shade, use sunscreens with a high sun protection factor (SPF), and in appropriate amounts.

Results and Discussion
The best advice is to avoid sun holidays where minimal clothing is worn, exposing large areas of the skin. All kinds of textile are effective in protecting the skin from UVR, but warm climates are a challenge when aiming to cover the whole body with clothing. Avoiding the sun entirely between 12:00 and 15:00 during the summer (summer time) will reduce the sun exposure considerably, as 50% of the UVR is present during these 3 hours of the day. When people are determined to expose themselves to sunlight, disregarding the mentioned advice, sunscreens must be used. Sunscreen should be applied before exposure to sunlight, and persons with photosensitivity diseases should use the highest possible SPF. Even more important is the amount of sunscreen used to achieve a proper protection. Research has shown that two consecutive applications of sunscreen before sun exposure, with an interval of 15-20 minutes between applications, will provide the optimal protection throughout the day. The protection will be close to that of the SPF labelled on the sunscreen container, except on the person's back and back of the legs. A basic protection against UVR and blue light can be obtained by sunless tanning products which provide a SPF of 2-4 for approx. 5-7 days. Sensitivity to blue light is often seen in solar urticaria where antihistamine in high doses is advisable in combination with the other mentioned protection measures. Treatment of the photosensitivity diseases may be necessary, using systemic or topical glucocorticosteroids or other immunosuppressive drugs to reduce the risk of abnormal reaction to UVR.

Conflicts of Interest
None

References

Heerfordt IM, Torsnes LR, Philipsen PA, Wulf HC. Sunscreen use optimized by two consecutive applications. PLoS One 2018; 13(3): e0193916.

Faurschou A, Janjua NR, Wulf HC. Sun protection effect of dihydroxyacetone. Arch Dermatol 2004; 140(7): 886-7.

Faurschou A, Wulf HC. The relation between sun protection factor and amount of sunscreen applied in vivo. Br J Dermatol. 2007; 156(4): 716-9.

> IL054. Invited Lecture
Symposium MED-9 Photosensitivity diseases (Sally Ibbotson)

TOPICAL PHOTOALLERGY AND PHOTOPATCH TESTING

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Whilst the most common type of drug photosensitivity reaction is phototoxicity, photoallergy does less frequently occur and usually with topical drug delivery. Topical photoallergy requires initial sensitisation in order to subsequently elicit a delayed Type IV cell-mediated hypersensitivity reaction. Currently, the main culprits for topical photoallergy are sunscreen chemicals and non-steroidal anti-inflammatories. This reflects tonnage use, exposure patterns and the ability of individual drug types to elicit a photoallergic reaction. The investigation of choice for topical photoallergy is photopatch testing and whilst a European methodology has been established there are still potential variables in this technique. Refinement of the photopatch testing methodology is under investigation in a further European multicentre photopatch study, which is currently recruiting and will examine which are the most common culprits for topical photoallergy in the current clime and will also investigate the impact of irradiation timings within the photopatch test technique. Emphasis must be placed on the importance of this invaluable investigation and its availability within photodiagnostic or contact allergy centres of expertise, in order to thoroughly evaluate topical photoallergy.

> IL056. Invited Lecture
Symposium MED-9 Photosensitivity diseases (Sally Ibbotson)

PHOTOTHERAPY FOR THE PHOTOSENSITIVITY DISEASES

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The concept of idiopathic photodermatoses include dermatological diseases that occur in otherwise healthy individuals as a result of exposure to natural or artificial light without the intervention of an external photosensitizer. Phototherapy of polymorphic light eruption is based on the increased melanin formation and thickening of the stratum corneum and immunoregulation of the skin. A short course of radiation (12-15 sessions) is usually enough to achieve a hardening effect in many cases. NUVB desensitization is commonly preferred over PUVA due to safety and convenience reasons, independently of the light spectrum inducing the cutaneous disease. In solar urticaria, the aim of desensitization is to keep the patients in a chronic refractory state through repeated exposure of UV radiations. Again, NBUVB is commonly used independently of the wavelengths precipitating solar urticaria. UVA (without psoralen) or even solar light repeated exposure could be used in this disease. As clinical exacerbation and even extensive cutaneous disease may result in anaphylaxis, it is mandatory to determine the MUD prior to any procedure.

Phototherapy can also be used in the management of other photodermatoses like actinic prurigo, hydroa vacciniforme or chronic actinic dermatitis.

> OC016. Oral Communication
Symposium MED-9 Photosensitivity diseases (Sally Ibbotson)

HIGH LEVELS OF OXIDATIVELY GENERATED DNA DAMAGE 8,5′-CYCLO-2′-DEOXYADENOSINE ACCUMULATE IN THE BRAIN TISSUES OF XERODERMA PIGMENTOSUM GROUP A GENE-KNOCKOUT MICE

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Xeroderma pigmentosum (XP) is a genetic disorder associated with defects in nucleotide excision repair, a pathway that eliminates a wide variety of helix-distorting DNA lesions, including ultraviolet-induced pyrimidine dimers. In addition to skin diseases in sun-exposed areas, approximately 25% of XP patients develop progressive neurological disease, which has been hypothesized to be associated with the accumulation of an oxidatively generated type of DNA damage called purine 8,5'-cyclo-2'-deoxynucleoside (cyclopurine). However, that hypothesis has not been verified. In the present study, we tested that hypothesis by using the XP group A gene-knockout (*Xpa*$^{-/-}$) mouse model (1). To quantify cyclopurine lesions in this model, we previously established an enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody (CdA-1) that specifically recognizes 8,5'-cyclo-2'-deoxyadenosine (cyclo-dA) (2). By optimizing conditions, we increased the ELISA sensitivity to a detection limit of ~one cyclo-dA lesion/10^6 nucleosides. The improved ELISA revealed that cyclo-dA lesions accumulate with age in the brain tissues of *Xpa*$^{-/-}$ and of wild-type (wt) mice, but there were significantly more cyclo-dA lesions in *Xpa*$^{-/-}$ mice than in wt mice at 6, 24 and 29 months of age. These findings are consistent with the long-standing hypothesis that the age-dependent accumulation of endogenous cyclopurine lesions in the brain may be critical for XP neurological abnormalities.

References

1) H. Nakane et al., High incidence of ultraviolet-B- or chemical-carcinogen-induced skin tumours in mice lacking the xeroderma pigmentosum group A gene, Nature, 377 (1995) 165-168.
2) T. Iwamoto et al., Quantitative and *in situ* detection of oxidatively generated DNA damage 8,5\'-cyclo-2\'-deoxyadenosine using an immunoassay with a novel monoclonal antibody, Photochem Photobiol, 90 (2014) 829-836.

> IL060. Invited Lecture
Symposium MED-10 Dentistry (Ellen Bruzell)

LIGHT SOURCES AND LIGHT-DEPENDENT PROCEDURES IN DENTISTRY

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Dental personnel encounter a number of optical sources emitting a broad spectrum of wavelengths. Along with the usefulness, to various extents, of optical radiation in treatment and diagnostics, comes the risk of radiation exposure to the operator's eyes and the patients' oral tissues. The presentation will cover the "involuntary" and "voluntary" use of different light sources. The curing light used for photopolymerisation of dental materials is an everyday procedure in the clinic. Curing lights have evolved from emitting moderate intensities of UV to high intensity blue light LEDs of several watts per centimeter squared.

Dental bleaching procedures combined with light is a controversial topic. It is debated whether the light improves the efficiency. Thereby, the justification of the use is questionable. The bleaching lights may emit UV or blue light or both in doses that can exceed limit values. The light sources are diverse: halogen, LED, and lasers among others.

Lasers are used in a wide variety of applications depending on their optical characteristics. Some laser procedures are established, the use of others are being debated, and some are under development.

PDT to treat infections and oral lesions is a recent treatment option, and the applications that are under development can use several light sources. PDT protocols vary greatly between e.g. photosensitiser type and application time, light sources, and light dose. Examples will be given from our research group's use of formulated curcumin, lumichrome and porphyrins.

The use of light in dental diagnostics of caries and oral cancer could be a valuable tool, but is not used by most dentist. Imaging of bacterial infections and lesions may be an aid in e.g. experimental PDT and toxicology.

Finally, illumination sources are important for optimal viewing conditions for dental personnel. The operating lights have increased intensity compared to previous versions, and the use of lights on loupes and in microscopy have increased. Can it be too much light?

> IL061. Invited Lecture
Symposium MED-10 Dentistry (Ellen Bruzell)

ATOMIC FORCE MICROSCOPY (AFM) STUDY OF THE PHOTODYNAMIC EFFECTS ON ENTEROCOCCUS FAECALIS BIOFILMS

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Microbial biofilms are related in most pathologies in the oral cavity. The elimination of this bioflim will therefore be an important part of the different treatments that dentist shoul do in differents oral pathologies. In our study we used atomic force microscopy (AFM)to visualize injures and to determine surface roughness, as well as confocal laser scanning microscopy (CLSM) to enumerate live and dead bacterias, to determine the effeects of photodinamic therapy on the *Entorococus faecalis* bioflims.

In this study we have used two different light source toproduce photodinamic therapy; a 630nm LED light and 670nm laser light. To achieve the photodinamic effect two differents dyes were used; Blu Toulidine (with the 630nmLED) and the methylene blue (with the 670nm laser).

AFM images showed PDT with methylene blue and a 670-nm diode laser (output power 280 mW during 30 s) and toluidine blue and a 628-nm LED light (output power 1000 mW during 30 s) induced severe damage, including cell lysis, to E. faecalis biofilms, with the former also causing an important increase in surface roughness. These observations were confirmed by the increase in dead cells determined using CLSM. Our results highlight the potential of PDT as a promising method to achieve successful oral disinfection.

> IL062. Invited Lecture
Symposium MED-10 Dentistry (Ellen Bruzell)

PHOTODYNAMIC TREATMENR (PDT) OF ORAL LICHEN PLANUS (OLP)

Authors: Juliane Hesse[1], Sigrid I. Kvaal[1]
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1) Institute of Clinical Dentistry, Faculty of Dentistry,, University of Oslo

Introduction
Lichen planus is an autoimmune skin disease, which also affects the mucous membranes of the oral cavity, oesophagus and genitalia. On the skin, it presents with itchy scaly plaques, which burn out on average after 3 years. In the mouth OLP present in different grades of severity from symptom free white stripes to painful recurrent ulcers. Eating and drinking, especially spicy foodstuff, is painful and has considerable influence on the quality of life. Females are affected twice as often as males and the incidence is reported to be from 2-4%. The disease might last up to 20 years. It is regarded as a pre-malign lesion. There is no known cure for the disease and potent cortisone is prescribed to relieve symptoms. In some countries a mouth gel is available on prescription, but this drug delivery is not available in all countries(1).

Method
PDT in the oral cavity was performed with application of methyl 5-aminovulinate (MAL) [Metvix®) as photo-sensor (PS) to the treated area which was covered for 15 mins and repeated after one hour. Three hours after initiation of treatment a radiant exposure of 75 J/cm^2 of red light in the region 600 – 660 nm was delivered to the affected area at irradiances of 100 – 130 mW/cm^2 using a light-emitting diode (LED) light source(2).

Results
MAL-PDT was shown to be effective in reducing the area affected by the disease as well as reducing the pain. The time between new ulcers was longer and they were less painful. The results of MAL-PDT seemed to last for long periods, up to several years. Experience shows that OLP affected gingiva are more resistant to treatment(2).

Discussion
The thin epithelium allows easy penetration of Metvix®. However not all areas can be covered and wash-out by saliva may be a problem. Most patients experienced some pain during and post MAL-PDT, indicating that there had been a tissue reaction. Results showed improvement both on the treated side as well as the non-treated side, but none resulted in complete healing. However, there was a longer time between exacerbations, and the ulcers lasted a shorter time and were less painful. Both improved oral hygiene and reduction in superinfections may also contribute to patients experiencing less oral discomfort. Clinical experience has shown that there are treatment resistant forms of OLP and the reason for this is still unknown. A new project is planned to compare MAL-PDT with treatment with potent cortisone – standard recommended treatment for painful OLP

Conclusions
MAL-PDT is effective in reducing signs and symptoms in some patients with OLP and the improvement seems to be long-lasting.

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Conflict of interest
None

References

1. Gupta S, Jawanda MK. Oral Lichen Planus: An Update on Etiology, Pathogenesis, Clinical Presentation, Diagnosis and Management. Indian J Dermatol. 2015;60(3):222-9.
2. Kvaal SI, Angell-Petersen E, Warloe T. Photodynamic treatment of oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;115(1):62-70.

> IL063. Invited Lecture
Symposium MED-10 Dentistry (Ellen Bruzell)

LIGHT-BASED ANTIMICROBIAL APPROACHES IN DENTISTRY – CHANCES AND LIMITATIONS

Authors: Fabian Cieplik[1]
Presenting Author: Fabian Cieplik
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The 2016 Review on Antimicrobial Resistance has predicted that the number of annual deaths attributable to antimicrobial resistance will increase globally from the current 700,000 to 10 million in the year 2050 if no appropriate action is taken immediately. In light of this increasing threat of resistance towards conventional antibiotics and antiseptics, alternative antimicrobial approaches are desperately needed. In particular in the field of dentistry, where usually no life-threatening diseases need to be treated, it seems reasonable to promote research for suchlike alternatives. In this context, especially light-based approaches including antimicrobial photodynamic therapy (aPDT), photothermal therapy (PTT) or low-level light (or laser) therapy (LLLT), have increasingly been proposed in the last two decades.

This talk aims to give an overview about these approaches by summarizing evidence from in vitro studies as well as recent clinical trials and to discuss the chances and limitations for application of light-based antimicrobial approaches in dentistry.

> IL064. Invited Lecture
Symposium MED-10 Dentistry (Ellen Bruzell)

ANTIBACTERIAL PHOTODYNAMIC THERAPY FOR TREATMENT OF ORAL BIOFILMS RELATED TO PERIODONTAL AND PERI-IMPLANT DISEASES

Authors: Håkon Valen[1]
Presenting Author: Håkon Valen
1) Nordic Institute of Dental Materials

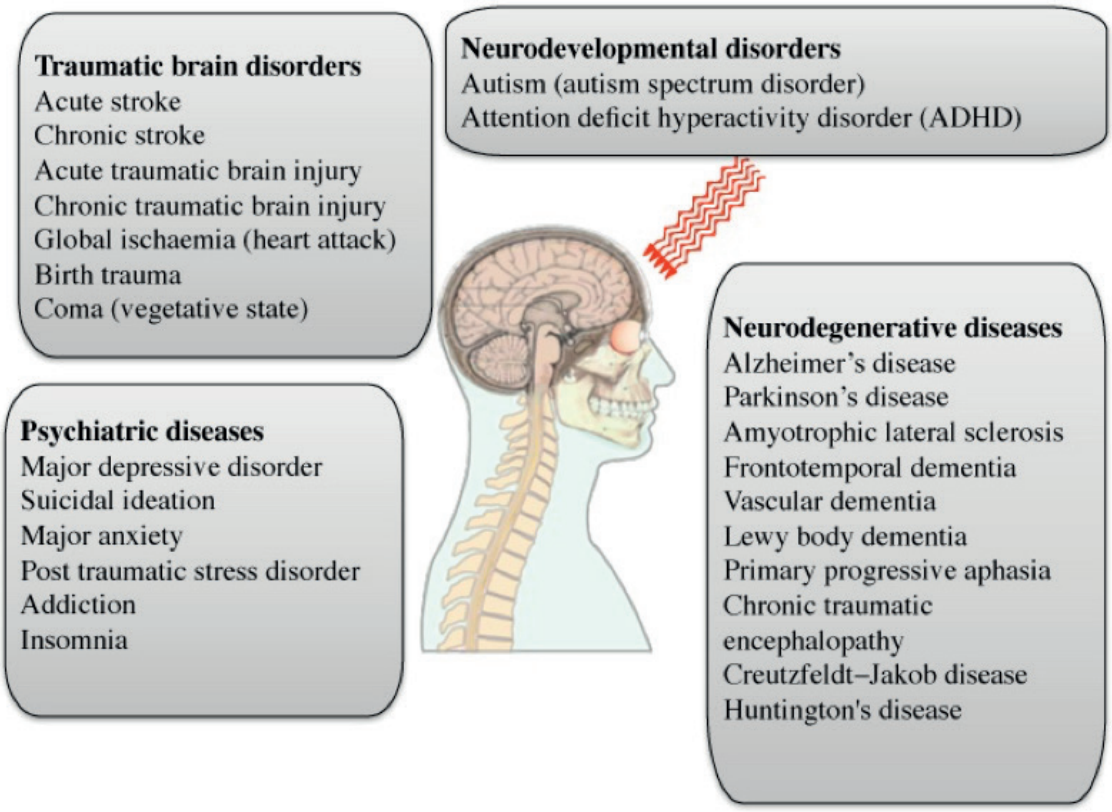
Oral biofilm formation around teeth and dental implants may cause inflammation of the surrounding tissue, which may lead to breakdown of the attachment of the tooth or implant. Ultimately, if left undisturbed it may lead to loss of tooth or implant. At present the gold standard for treatment of periodontal disease is mechanical disruption and removal of the biofilm with scaling and root planning and training of patients in control of biofilm levels compatible with health for the individual. The mechanical treatment does not remove all bacteria associated with periodontal disease, therefore different adjunctive treatment modalities are suggested. Antibacterial photodynamic therapy is one such treatment strategy. This talk will discuss current concepts for antibacterial photodynamic therapy related to oral biofilms and periodontal and peri-implant diseases. What are the clinical difficulties and how can we improve efficacy of antibacterial photodynamic therapy regarding both photosensitizers used in liquid solutions and on material surfaces.

> IL065. Invited Lecture
Symposium MED-11 Photobiomodulation (Michael Hamblin)

PHOTOBIOMODULATION FOR THE BRAIN: HAS THE LIGHT DAWNED?

Authors: Michael R. Hamblin[1949]
Presenting Author: Michael R. Hamblin
1) Massachusetts General Hospital

Photobiomodulation (PBM) describes the use of red or near-infrared light to stimulate, heal, regenerate, and protect tissue that has either been injured, is degenerating, or else is at risk of dying. One of the organ systems of the human body that is most necessary to life, and whose optimum functioning is of most concern to humans in general, is the brain. The brain suffers from many different disorders that can be classified into three broad groupings: sudden events (stroke, traumatic brain injury, and global ischemia), degenerative diseases (dementia, Alzheimer's and Parkinson's), and psychiatric disorders (depression, anxiety, post traumatic stress disorder, autism). There is some evidence that all these seemingly diverse conditions can be beneficially affected by applying light to the head. There is even the possibility that PBM could be used for cognitive enhancement in normal healthy people. In this transcranial PBM (tPBM) application, near-infrared (NIR) light is often applied to the forehead because of the better penetration (no hair, longer wavelength). Some workers have used lasers, but recently the introduction of inexpensive light emitting diode (LED) arrays has allowed the development of light emitting helmets or "brain caps". Transcranial LED light sources are ideally suited to be home use devices. This review will cover the mechanisms of action of photobiomodulation to the brain, and summarize some of the key pre-clinical studies and clinical trials that have been undertaken for diverse brain disorders.



> IL066. Invited Lecture
Symposium MED-11 Photobiomodulation (Michael Hamblin)

ESTABLISHING PHOTOBIOMODULATION (PBM) THERAPY AS A FIRST-LINE MEDICAL TREATMENT FOR ORAL MUCOSITIS.

Authors: James D. Carroll[1]
Presenting Author: James Carroll
1) THOR Photomedicine Ltd

Objective
To draw a roadmap establishing Photobiomodulation (PBM) Therapy as a first-line medical treatment for oral mucositis.

Background
Oral Mucositis (OM) is a severe side effect of radiotherapy, high dose chemotherapy and Hematopoietic Stem Cell Transplantation (HSCT). Photobiomodulation (PBM Therapy) previously known as Low-Level Laser Therapy (LLLT) is effective in reducing and even preventing side effects.

Results so far
1992 Ciais et al. Publish the first report on “laser therapy” for OM

1997 Cowen et al. published the first double-blind randomised trial

2010 Bjordal et al. First systematic review of eleven OM RCTs

2013 The Multinational Association of Supportive Care in Cancer recommend PBM.

2014 Bezinelli et al. show PBM reduces costs by 30%

2016 The American insurance company Blue Cross Blue Shield declare “medically necessary for the prevention of oral mucositis in select patients.”

2017 Antunes et al. Show PBM improves long term survival of OM patients

2017 The NHS fund clinical trials across eight UK hospitals

2018 The UK National Institute of Health and Care Excellence (NICE) recommend PBM for prevention and treatment of OM

2019 At least Forty-two clinical trials have been published, and more are in progress.

Barriers
How many more trials are needed? What is missing?

In the United States, there is no FDA code for PBM, and there is limited insurance reimbursement. In Brazil, it is an accepted treatment by dentists, but there is no insurance reimbursement and a shortage of dentists in the public hospital to deliver PBM treatment. In the UK, there are no hospital treatment codes, and cost benefits have not yet been established in an NHS setting. Criticism of the current research includes poor recording and reporting of irradiation parameters and dose, lack of dose-response data, lack of key opinion leader support, lack of publication in high impact factor cancer journals.

Conclusion
PBM is safe, effective and reduces the cost of care and may even improve survival. Future papers need to be large multi-centre institutional clinical trials that include key opinion leaders as authors. Optical engineers or physicists must be involved in recording and reporting irradiation parameters and dose. Publish in high impact factor cancer journals to get into national guidelines.

> IL067. Invited Lecture
Symposium MED-11 Photobiomodulation (Michael Hamblin)

THE POTENTIAL OF PHOTOBIOMODULATION TO ENHANCE ANGIOGENESIS AND WOUND HEALING

Authors: Lisa Karner[1], Eleni Priglinger[1], Julia Maier[1], Raimund Winter[2], Kurt Schicho[2], Sabrina Rohringer[1], Wolfgang Holnthoner[1], Peter Dungel[1]
Presenting Author: Peter Dungel
1) Ludwig Boltzmann Institute for Experimental and Clinical Traumatology 2) Department of Plastic, Aesthetic and Reconstructive Surgery, Medical University of Graz, Austria

The application of light in various therapeutic settings known as Photobiomodulation (PBM) is well established. Typical indications for PBM are the improvement of wound healing and tissue regeneration, scarring, and perfusion as well as pain therapy. Tissue perfusion is mandatory for successful wound healing. Nevertheless, there is a lack of mechanistic studies.

Endothelial cells and stem cells are key factors in angiogenic processes. Endothelial cells are typically isolated from human umbilical vein. A highly interesting source for adult stem cells is adipose tissue, from which the stromal vascular fraction (SVF), a heterogeneous cell population including the adipose-derived stromal/stem cells (ASC), can be obtained.

PBM of different wavelengths was tested for stimulatory effects on regenerative potential as well vasculogenesis. Pulsed blue (475 nm), green (516 nm) and red light (635 nm) from light-emitting diodes by REPULS were applied on HUVEC and freshly isolated SVF. Cell phenotype, cell number, viability, adenosine triphosphate content, cytotoxicity and proliferation, but also osteogenic, adipogenic and pro-angiogenic differentiation potential were analysed.

The colony-forming unit fibroblast assay revealed a significantly increased colony size after PBM with red light compared to untreated cells. PBM with green and red light resulted in a stronger capacity to form vascular tubes by SVF when cultured within 3D fibrin matrices compared to untreated cells, which was corroborated by increased number and length of the single tubes and a significantly higher concentration of vascular endothelial growth factor. Similar positive effects on proliferation and vasculogenesis could be reproduced in endothelial cells and in co-cultures of these cell types.

In a subsequent second study we tested the most promising setting of PBM in a more complex chick egg chorioallantoic membrane (CAM) assay. Chick embryos were cultured in steril conditions until day 10 and subjected to PBM with pulsed red light (635nm). Daily incident light microscopic photo-documentation was performed. The number of neovascular branches was analyzed in randomized pictures of the CAM assays. Also in this model pulsed red light increased the number of vessel junctions in defined regions of interest.

Our studies confirmed significant beneficial effects of PBM on vascularization potential and proliferation capacity both in various *in vitro* cell culture models as well as in the CAM model. Further studies have to focus on intracellular mechanisms induced by different wavelengths in order to optimize this promising therapy in tissue regeneration.

Supported by FFG grant Basisprogamm 853128.

> IL068. Invited Lecture
Symposium MED-11 Photobiomodulation (Michael Hamblin)

THE IMPACT OF PHOTOBIOMODULATION ON LIPID METABOLISM IN NEURONAL CELLS

Authors: Svitlana M. Levchenko[Shenz], Tymish Y. Ohulchanskyy[Shenz], Junle Qu[Shenz]
Presenting Author: Svitlana M. Levchenko
1) Shenzhen University

Introduction

The beneficial effects of photobiomodulation (PBM) in a wide range of neurological disorders have been demonstrated numerous times, using cell culture and animal models, and also in some clinical trials[1-3]. However, the precise mechanisms involved in the capability of PBM to relieve symptoms, slow down the progress and treat some brain disorders remain not clear. From the other standpoint, a new intriguing connection between lipid dysfunction and neurodegenerative disorders has been recently discovered [4-6]. Thus, modulation of lipid metabolism may provide new pathways for disease treatment or prevention.

This study was aimed to explore the effect of PBM on lipid metabolism in neuronal cells and examine whether it can be modulated by NIR light[7].

Methods

Here we employed fluorescence and CARS microscopies for real-time monitoring and quantitative analysis of the 808nm light effect on lipid metabolism and lipid droplets (LDs) formation in primary rat cortical neuronal cells. The cells were irradiated with an 808nm diode laser in the continuous wave mode with a power density of 50 mW/cm^2 for different time periods to deliver the irradiation doses of 0.3, 3, 10, and 30 J/cm^2.

Results and Discussion

Our data indicated noticeable dose-dependent changes in the average lipids level in neuronal cells after irradiation with 808nm laser light. Furthermore, a correlation between PBM induced ROS generation, the lipids level and lipid droplets formation in neurons was revealed.

Conclusions

We have for the first time demonstrated that irradiation with NIR light induces ROS mediated changes in lipid metabolism and causes LDs formation in neurons. Our findings can hopefully contribute to the development of therapeutic approaches for neurological disorders treatment via NIR light control of lipid metabolism in neuronal cells.

Acknowledgements

The authors are grateful to Prof. Paras N. Prasad, Dr. Andrey N. Kuzmin and Dr. Artem Pliss for providing equipment for this research and extensive discussions.

Conflicts of Interest

The authors have declared that no conflicts of interest exist.

References

[1] Hamblin MR *BBA Clin* **2016**, *6*, 113.
[2] Berman MH, Halper JP, Nichols TW, Jarrett H, Lundy A, Huang JH *J Neurol Neurosci* **2017**, *8*.
[3] Huang YY, Nagata K, Tedford CE, Hamblin MR *J Biophotonics* **2014**, *7*, 656.
[4] Liu L, Zhang K, Sandoval H, Yamamoto S, Jaiswal M, Sanz E, Li Z, Hui J, Graham BH, Quintana A, Bellen HJ *Cell* **2015**, *160*, 177.
[5] Liu L, MacKenzie KR, Putluri N, Maletic-Savatic M, Bellen HJ *Cell Metab* **2017**, *26*, 719.
[6] Sato N, Morishita R *Front Aging Neurosci* **2015**, *7*, 199.
[7] Levchenko SM, Kuzmin AN, Ohulchanskyy TY, Pliss A, Qu J, Prasad PN *ACS Chem Neurosci* **2018**.

> IL069. Invited Lecture

Symposium MED-11 Photobiomodulation (Michael Hamblin)

PHOTOBIOMODULATION AS A SUPPORTING HIGH CARIES RISK THERAPY

Authors: Lidija Nemeth[1, 2], Ksenija Cankar[3], Maja Grošelj[1, 2], Helena Ban Frangež[4], Igor Frangež[5]
Presenting Author: Lidija Nemeth
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Introduction

Dental caries is a complex multifactorial chronic infectious disease influenced by several risk or protective factors. Saliva has an important role in both, caries and the remineralization process. Caries risk assessment is defined as the probability of new caries lesion development or the existing lesion progression in a given time period. Caries therapy consists of clinical diagnostics and risk factors assessment, which are followed by targeted elimination of risk factors and less restorative, but abundant preventive therapeutic measures. The aim of our prospective randomized study was to elucidate how photobiomodulation of major salivary glands with polychromatic light or LED light affects caries risk factors in high caries risk patients. The study was approved by Republic of Slovenia National Medical Ethics committee (No. 0120-539/2016-2 KME 40/11/16).

Methods

Thirty-six high caries risk patients according to Cariogram [1] were randomly assigned to one of three experimental groups: the first, irradiated with polarized polychromatic light (40 mW/cm^2, wavelengths 480 - 3400 nm); the second, irradiated with LED light in a continuous mode (16 mW/cm^2, wavelengths 625 nm, 660 nm and 850 nm); the third, irradiated with same LED light in a pulsed mode. The fourth group was the control, for which a non-therapeutic visible light was used. The light was administered transcutaneous extra orally bilaterally above the parotid and submandibular glands for 10 minutes and intra orally above the sublingual glands for 5 minutes, 25 minutes cumulative per session, 3 times a week, for 4 consecutive weeks. Each patient\'s caries risk was assessed according to Cariogram before and after therapy. Caries risk factors were determined from samples of collected saliva before the irradiation, two weeks after it commenced, at the end and 4 weeks after the end of the irradiation therapy.

Results

At the end of treatment: in group, irradiated with polarized polychromatic light, and in group, irradiated with continuous LED light, the Streptococcus mutans and Lactobacillus count decreased and salivary buffering capacity increased (one-way repeated measures ANOVA, Dunnett's test, $p< 0.05$). In group, irradiated with pulsed LED light Streptococcus mutans counts decreased, unstimulated salivary flow and salivary buffering capacity increased (one-way repeated measures ANOVA, Dunnett's test, $p< 0.05$). In all three experimental groups, caries risk was lower (Wilcoxon test, $p< 0.05$). In placebo control group, there were no statistically significant differences between parameters before and after therapy.

Conclusion

We concluded that photobiomodulation of major salivary glands in high caries risk patients can reduce the cariogenic bacteria in saliva and improve some salivary parameters, thus may be useful as one of the supporting therapies with the effect of reducing overall caries risk.

Conflicts of interest

The manuscript represents valid work. We have no conflicts of interest to declare.

Reference

[1] Bratthall D, Hansel Petersson G. 2005. "Cariogram--a multifactorial risk assessment model for a multifactorial disease." Community Dent Oral Epidemiol 33(4):256-64

> IL070. Invited Lecture
Symposium MED-11 Photobiomodulation (Michael Hamblin)

THE EFFECT OF PHOTOTHERAPY WITH LIGHT EMITTING DIODES ON CHRONIC WOUND HEALING

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Presenting Author: Ksenija Cankar
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Aim
Phototherapy is one of the possible adjuvant methods to the standard treatment of the wounds. The aim of our randomized, double blind study was to verify if phototherapy with light emitting diodes (LED) as an adjuvant therapy improves healing of the chronic wounds.

Materials and methods
Forty patients with diabetes and 39 without diabetes that were due to chronic wound were randomized into study group that received besides standard treatment protocol also phototherapy with LED (wavelengths 625 nm, 660 nm and 850 nm and power density 2.4 J/cm^2, three times weekly for 8 weeks) and into control group that besides standard treatment received placebo therapy (light therapy between 580 and 900 nm and power density 0.72 J/cm^2). Effects on healing was evaluated with clinical classification of the wound bed according to Falanga scale, measuring wound surface using computer program Image J and evaluation of the microcirculation with Laser Doppler flow.

Results
Wounds of the patients in the study group were according to Falanga scale healing faster in diabetic and in non diabetic patients; diabetics LED vs. diabetics placebo; p=0.0005 and non-diabetics LED vs. non-diabetics placebo; p=0.0014. Therapy with LED did not significantly influence on wound shrinkage in any of the groups. Microcirculation was significantly improved in both groups receiving LED therapy (diabetics; p=0.033, non-diabetics; p=0.040), while in control groups remained the same.

Conclusions
Phototherapy with LED as an adjuvant therapy significantly improved healing of chronic wounds (faster granulation and improved microcirculation) in diabetic and in non-diabetic patients.

> OC017. Oral Communication
Symposium MED-11 Photobiomodulation (Michael Hamblin)

BIOMODULATION: A BONUS IN PERIODONTAL TREATMENT

Authors: Beatrijs Deruyter[1]
Presenting Author: Beatrijs Deruyter
1) THOR

Introduction

Periodontitis and periimplantitis, both proven to contribute and/or exacerbate systemic diseases by their inflammatory response, are a concern in general health care. Tissue repair and regeneration versus damage caused by this inflammation, will depend on the redox state of the tissue. Polymorphonuclear cells, body's first defence system are playing an important role by its triple mechanism (Opsonisation, Free radicals & Neutrophil Extracellular Traps)(1). In this complex inflammatory mechanism, mainly caused by detrimental microbiome, free radicals, like Reactive Oxygen Species(ROS) are causing the production of NO, binding on the last enzyme (cytochrome C oxidase) of the oxidative phosphorylation in the mitochondria. This has its negative effects on the redox state of cell, tissue and ATP production. Finding a cost-effective and repeatable treatment without causing detrimental side effects, are a necessity.

The preservation of cementum and Enamel Remnants of Malassez (ERM) is crucial in this concept since they produce Enamel Matrix Proteins (EMP), signalling proteins for regeneration(2). Free running pulsed lasers, by its very short burst of energy may be contributing to new regenerative treatment possibilities. By choosing the right wavelength and energy settings we create thermal interaction with enough thermal relaxation in respect to the surrounding tissues. They are capable of restructuring and disinfecting the dentine, cold ablation of the infected pocket lining and by dissipation of the energy, cause coagulation with release of the associated growth factors, destroy inflammatory enzymes and pathogens and finally cause bio-modulation, with interaction on the redox state(3, 4). Near Infra Red wavelengths are capable at low energy levels to break bonding of NO on cytochrome C oxidase, promoting wound healing, tissue protection and stimulate growth factors in the compromised tissue(5).

Materials and Methods

Fifty-three patients having dpsi 3+ or 4, undergoing all the same protocol. Assessment was based on following parameters: pocket depth measurement (PD),bleeding on probing(BOP) recession, panoramic X-Ray and microbiological assessment with real time Polymerase Chain Reaction (rPCR). Antibacterial medication was only administered on advice of the bacterial assessment. Treatment protocol consists of a true OFMD of scaling with adjunct of FRP lasers.

Results

Compared to baseline, at 2, 8 up to 14 months, without retreatment. Clinical outcome of the parameters for all cases were statistically significant improved to baseline.

References

1. Cooper PR, Palmer LJ, Chapple IL. Neutrophil extracellular traps as a new paradigm in innate immunity: friend or foe? Periodontology 2000. 2013;63(1):165-97.
2. Bosshardt DD. Are cementoblasts a subpopulation of osteoblasts or a unique phenotype? Journal of dental research. 2005;84(5):390-406.

> OC018. Oral Communication
Symposium MED-11 Photobiomodulation (Michael Hamblin)

PHOTOBIOMODULATION AT 660 NM ACCELERATES WOUND HEALING VIA THE JAK/STAT CELLULAR PATHWAY

Authors: Heidi Abrahamse[1]
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Keywords
Photobiomodulation, Diabetes mellitus, growth factors, JAK, STAT, proliferation, wound healing

Introduction
Diabetic patients have a higher probability of developing chronic ulcers, which are a major cause of non-traumatic limb amputations and reduced quality of life. Cell proliferation, differentiation and migration is critical for physiological outcomes including wound repair. Diabetic ulcers present with reduced growth factor production that affect healing, including fibroblast migration and proliferation. Activation of the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signalling pathway results in transcription and downstream events such as cellular proliferation and migration, and is activated by epidermal growth factor (EGF) which is reduced in cases of diabetes. Photobiomodulation (PBM) involves exposing wounds to light emitting diodes (LED) or lasers, and has been shown to stimulate cellular migration and proliferation. However, the mechanism/s involved in these observations are not well understood. The aim of this investigation was to determine if PBM activates the JAK/STAT signalling pathway leading to cellular migration and proliferation.

Methods
Normal, wounded, diabetic and diabetic wounded human fibroblasts (WS1; ATCCÒ CRL-1502Ô) were irradiated once with a 660 nm diode laser (100 mW, 11 mW/cm^2, area 9.1 cm^2, 5 J/cm^2, 454 s). Exogenous rhEGF treated and non-irradiated (0 J/cm^2) cells served as controls. Cells were incubated for 48 h post-irradiation. Cellular migration rate (microscopy), proliferation (BrdU), and EGF expression, phosphorylated (p-)EGF receptor (p-EGFR), p-JAK2, p-STAT1 and p-STAT5 (ELISA) was determined.

Results
PBM at 660 nm with 5 J/cm^2 significantly increased wound migration rate in wounded and diabetic wounded cells. Proliferation was significantly increased in all cell models. Expression of EGF, and activation (phosphorylation) of EGFR, JAK2, STAT1 and 5 were all increased.

Conclusion
PBM of wounded and diabetic wounded cells in vitro at 660 nm with 5 J/cm^2 stimulates migration and proliferation of cells via expression of EGF which binds to and phosphorylates EGFR which in turn leads to activation of the JAK/STAT pathway.

References

1. Jere S.W., Houreld N.N. and Abrahamse, H. (2018) Photobiomodulation at 660 nm stimulates proliferation and migration of diabetic wounded cells via the expression of epidermal growth factor and the JAK/STAT pathway. Journal of Photochemistry & Photobiology, B: Biology 179:74–83. Impact Factor 4.2
2. Jere S.W., Houreld N.N. and Abrahamse, H. (2017) The JAK/STAT signaling pathway and photobiomodulation in chronic wound healing. Cytokine & Growth Factor Reviews 38:73-79 Impact Factor 6.74

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Conflicts of Interest
The authors declares no conflict of interest.

> P019. Poster

Symposium MED-11 Photobiomodulation (Michael Hamblin)

LOW-LEVEL LIGHT THERAPY FOR FRONTAL FIBROSING ALOPECIA. A RANDOMIZED CLINICAL TRIAL

Authors: Pablo Fonda Pascual[1, 3], David Saceda Corralo[2, 3], Cristina Pindado Ortega[2], Francisco de Alcántara Nicolás[1], Santiago Vidal Asensi[1], Pedro Jaen Olasolo[2, 3], Sergio Vañó Galván[2,3]
Presenting Author: Pablo Fonda Pascual
1) Hospital Central de la Defensa. Madrid, Spain 2) Ramon y Cajal University Hospital. Madrid, Spain 3) Instituto de Investigación Sanitaria R

Introduction and Objectives

Frontal fibrosing alopecia (FFA) is a lymphocytic scarring alopecia with a rising prevalence in Dermatologic clinics. Available therapies are not greatly effective in order to interrupt disease progression and symptoms are difficult to treat. Furthermore there are not any randomized controlled trials involving this disease. Low level-light therapy (LLLT) has demonstrated its effectiveness in androgenetic alopecia and it may even play some positive role in scarring alopecias such as lichen planopilaris.

In patients with FFA we evaluated the anti-inflammatory, antifibrotic efficacy of domiciliary LLLT to control disease and symptomatology.

Methods

We designed a single-centre, double-blinded, and randomized clinical trial. Helmet-shaped devices composed by 246 high-powered red LEDs at a wavelength of 630 nm were given to patients and used 15 minutes daily for 6 months. Each device had a sham side and an active side, and the latter was masked and randomized for all 37 patients. The active side emitted at a fluence of 4,5 J/cm^2 whereas sham side was 10 times weaker at a fluence of 0,45 J/cm^2. Patients were evaluated at baseline and each 12 weeks for a total duration of 6 months.

The primary endpoint was the effect of LLLT in the disease, assessed with frontal regression and cicatricial band (in centimetres). Other primary endpoints included improvement in inflammatory clinical-trichoscopical (erythema, hyperkeratosis) and symptom-related variables in a qualitative scale (none/moderate/severe). Secondary endpoints included improvement in terminal and general hair thickness, assessed in each visit by a digital videodermatoscope with a trichoscopic software tool. We also evaluated the improvement in FFA severity scale (FFASS) and in its inflammation item. Patients underwent a patient global assessment (PGA) survey in each visit from 1 to 5.

Results

We herein report preliminary data of LLLT effectiveness after 6 months of therapy without comparing to placebo. Thirty-five patients completed treatment. Mean age was 63,26 years (range 49-81). After 6 months of LLLT there were global statistical differences with a worsening in mean frontal regression (8,68 cm at baseline versus 9,03 cm; p< 0,001), with no differences in the cicatricial band (p=0,882). There were significative decreases in pruritus (p=0,002), burning (p=0,013) and erythema (p< 0,001) with no differences in hyperkeratosis after 6 months of therapy (p=0,827).

Overall, there was a significant thickening of terminal hairs (p=0,048) but not a general hair thickening. There was not a significant reduction in FFASS global scale whereas the inflammation item showed a reduction after 6 months of therapy (p< 0,001). There were not differences in PGA score after therapy.

Conclusion

LLLT could be an effective therapy for symptom control and inflammation in FFA. Final comparative results will be presented in the next International Congress on Photobiology

> P020. Poster

Symposium MED-11 Photobiomodulation (Michael Hamblin)

REGULATORY ACTION OF LOW LEVEL LASER RADIATION OF NEAR INFRARED SPECTRAL REGION ON HYDROBIONTS

Authors: Aliaksandr Mikulich[1], Vitaly Plavsky[1], Nikolai Barulin[2], Sergey Rahautsou[2], Aliaksandr Vodchits[1], Inna Khodasevich[1], Ludmila Batay[1], Antonina Tretyakova[1], Ludmila Plavskaya[1], Tatsiana Ananich[1], Valentin Orlovich[1], Tien Quoc Tran[3], Cong Quang Tong[3], Ihar Leusenka[1]
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The effect of low level laser radiation on biological objects has been studied in a wide range of organisms. However, the question about acceptors, which define regulatory action of low level laser radiation on living organisms, is still the subject of hot discussions. The problem becomes more complicated when using IR laser radiation located outside the electron absorption bands of the main chromophores.

In this work, we studied the biological effect and possible acceptors of red and near IR laser radiation using zooplankton (branchiopod crustaceans) *Artemia salina L*. as a model system.

Influence on cysts was carried out by laser radiation with wavelengths of 635 nm, 808 and 976 nm (diode lasers); 1064 and 1342 nm (diode pumped Nd:YVO_4 laser); 1176 nm (diode pumped Nd:YVO_4 laser (1064 nm) with intracavity Raman self-frequency conversion). A percentage of hatching of *Artemia salina L*. nauplii from cysts (protective shell) (γ, %) after activation of eggs in salt water under conditions of the stable thermal regimen was chosen as a test to characterize the effect of laser radiation. The power density P = 3 mW/cm^2 was used throughout the study.

The studies have shown that depending on wavelength of acting radiation both stimulating and inhibiting effects are observed upon exposure to laser radiation. So, if the exposure to radiation with λ = 635 nm, λ = 976 and λ = 1064 nm has an inhibitory effect on the hatching of the nauplii, the radiation with λ = 808 nm, λ = 1176 nm and λ =1342 nm – stimulatory effect. The obtained dose curves are characterized by the presence of a pronounced extremum and not described by an exponential function. That points to the regulatory nature of biological effect. Since the laser radiation with λ = 808, 976, 1064, 1176, 1342 nm is outside the absorption band of porphyrins, the possible role of photosensitized reactions involving them should be excluded. Severe photobiological effect when exposed to radiation λ = 1176, 1342 nm can also questioned the role of the direct photochemical reactions of oxyhemoglobin (and other macromolecules containing the prosthetic groups), as the impact of a powerful pulsed laser radiation with a wavelength λ = 1060 nm to its solutions does not cause any reversible or irreversible spectral changes.

We believe that among possible acceptors of optical radiation of near infrared spectral region (at least on some of mentioned wavelengths) can be molecular oxygen. Biological activity of laser radiation can be explained by direct triplet-singlet excitation of molecular oxygen dissolved in biological tissues and its subsequent influence, as a signal (trigger) molecule, on physiological processes. Besides, water can be acceptor of radiation because absorption of aqueous solutions of biological molecules is entirely explained by absorption of solvent in region of λ = 1200 – 2500 nm.

The work was supported by Belarusian Republican Foundation for Fundamental Research, grant № F18VG-001.

> P021. Poster

Symposium MED-11 Photobiomodulation (Michael Hamblin)

MECHANISM OF ACTION OF LASER RADIATION AND CONSTANT MAGNETIC FIELD ON FISH SPERM

Authors: Vitaly Plavsky[1], Nikolai Barulin[2], Sergey Bushuk[1], Aliaksandr Mikulich[1], Ihar Leusenka[1], Sergey Rahautsou[2]
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It is generally believed that the joint (simultaneous) effect of laser radiation and a constant magnetic field is characterized by a synergistic effect and underlies the method of magneto-laser therapy. However, the biophysical mechanisms of this phenomenon have not been studied well.

To assess the effects of laser radiation and constant magnetic field, determination of penetration depth of laser radiation with λ = 543 nm into layer of fish sperm in absence and presence of constant magnetic field of 50 mT was carried out using confocal laser scanning microscopy. The velocities of fish spermatozoa after activation by water, percentage of motile spermatozoa, activity of enzymes consisting the spermatozoa were used as tests to determine the influence of mentioned physical factors on the functional and biochemical activity of sperm.

It is shown that application of constant magnetic field of 50 mT affects the penetration depth of laser radiation into multilayered tissue of living spermatozoa. The modifying effect of magnetic field on the depth of penetration of laser radiation into the tissue of spermatozoa can be due to a change in the structure of cells under the action of this physical factor.

It is established that the application of magnetic field of 50 mT changes the motion dynamics of spermatozoa: alterations in straight-line and curvilinear velocities induced by magnetic field, in the nature of trajectories of motion as well as a pronounced effect of the magnetic field on the distribution of cells according to the velocities are observed. This effect is a confirmation of the liquid crystalline nature of the structure of spermatozoa.

It is shown that the preliminary exposure of sperm to both laser radiation and constant magnetic field influences the functional and biochemical activity of sperm.

The maximal stimulating effect on the functional and biochemical parameters of sperm is observed when exposed to linearly polarized radiation; the photobiological effect induced in the same dose interval by natural light is much less pronounced. The magnitude of the stimulating effect of circularly polarized radiation takes an intermediate value.

The results obtained point to the synergism of the action of laser radiation and magnetic field. Among the photophysical processes of resonant and non-resonant nature (orientational effect of light, action of gradient forces, dipole-dipole interactions, thermo-optical processes), capable of causing photobiological effects, the determining role in the processes studied in this work belongs to the orientational effect of light on structures with liquid crystalline ordering character. The presence of weak absorption enhances the sensitivity of these systems to structural transitions induced by the orientational effect of polarized radiation.

This work was supported by Belarusian Republican Foundation for Fundamental Research, grant № F18VG-001.

> P176. Poster

Symposium MED-11 Photobiomodulation (Michael Hamblin)

A SYSTEMATIC REVIEW OF PHOTOBIOMODULATION FOR ORAL MUCOSITIS WITH A DOSE ANALYSIS

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Introduction

Photobiomodulation Therapy (PBM) formerly known as Low Level Laser Therapy (LLLT) is an effective treatment for reducing the incidence and severity of oral mucositis (OM) after high dose chemotherapy and/or radiotherapy. However, reported PBM irradiation parameters, dose per point, number of treatment points or treatment intervals vary widely.

Objectives

To systematically review randomized clinical trials (RCTs), summarise the PBM parameters and determine the range of effective treatment parameters.

Methods

Online databases were searched for RCTs comparing efficacy of PBM versus controls for prevention or treatment cancer therapy induced OM. Irradiation parameters and dose were reviewed for completeness and accuracy.

Results

44 controlled clinical trials were identified, 21 were excluded for lack of randomization, duplicate data, no placebo or insufficient treatment parameter data leaving 23 papers for review. The median scores: wavelength 660 nm (IQR 637-660), laser power 0.040 W (IQR 0.025-0.060), beam area 0.040 cm^2 (IQR 0.030-0.496), treatment time per point 28 secs (IQR 10-57), irradiance 1.0 (W/cm^2 0.2-2.1), energy dose 1.4 Joules (IQR 0.3-3.0), fluence dose 6.1 (J/cm^2 4.0-80.0).

Conclusions

No no one precise dose recommendation can be drawn due to a large variation on the reported data, but there is evidence of a dose window in the results. Dose and dose rate studies must be performed to identify optimal combination of treatment parameters.

> P177. Poster

Symposium MED-11 Photobiomodulation (Michael Hamblin)

THE EFFECT OF TISSUE THICKNESS AND SKIN COLOUR ON THE PENETRATION OF 850NM LED LIGHT TRANSMITTED THROUGH THE HUMAN CHEEK TO THE ORAL MUCOSA

Authors: Wayne J. Selting[2], James D. Carroll[1]
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Background

Oral Mucositis, is the most frequent complication of chemotherapy and HSCT treatment in cancer patients with an incidence in paediatric patients ranging from 52% up to 80%. To date, there is no standard therapy for mucositis management; the treatments are mainly supportive and palliative. There is growing evidence that Photobiomodulation is effective in both the therapy and prevention of mucositis.

Since lesions are located on the surface of the oral mucosa, the usual mode of application is intraoral. The ulcerations are extremely painful, and the simple task of retracting tissue to access the surface for treatment can be very painful.

Devices are now being developed to apply energy extra-orally requiring photons to pass through the skin and buccinator muscle of the cheek in order to reach the buccal mucosa. Reflection, scattering and absorption may make tissue thickness and skin colour significant factors affecting power density reaching the target tissue.

Objective

To measure the irradiance of 850nm LED light transmitted through cheek to the oral mucosa in patients with different Fitzpatrick skin types and different cheek thickness.

Materials and methods

42 patients-of-record from a private pediatric and orthodontic practice (32 children age 8 through 18, ten adults age 19 through 60) were recruited assigned a score based on skin pigmentation using the Fitzpatrick skin type scale and tissue thickness was recorded at the center of the cheek and 5mm from the commissure. An LED array consisting of 69 emitters (850nm, 65mw/cm^2) was applied to the external cheek. The power density of the energy passing through to the intraoral mucosa was recorded.

Results

A total of 506 measurements were taken, 63% of applied power density never penetrated the tissue, the average cheek thickness was = 6.6mm, and percentage transmission at 850 nm = 13.4% at the buccal mucosa

Conclusion

Penetration of therapeutic light is very strongly related to tissue thickness, and the relationship is logarithmic. Skin pigmentation did not significantly affect the power density of light transmission at 850nm and firm surface pressure decreases tissue thickness thereby enhancing light transmission to the buccal mucosa.

> IL071. Invited Lecture
Symposium MED-12 Synchrotron radiation (Peter Wobrauschek)

REVIEW: CHEMICAL IMAGING OF BIOLOGICAL SAMPLES AT THE MICRO- / NANOMETER SCALE USING SYNCHROTRON RADIATION

Authors: Peter Wobrauschek[1], Christina Streli[1]
Presenting Author: Peter Wobrauschek
1) TU Wien, Atominstitut

Chemical imaging is the capability of an analytical method to determine chemical elements, their spatial distribution and their time dependent changes within a sample of interest. Assuming the sample is prepared having a flat plane surface a set of data points can be chosen across this surface and is analyzed point by point getting the spectrochemical information by a suitable source and detector. In the case of X-ray fluorescence (XRF) typically lab sources as x-ray tubes combined with x-ray optical components as e.g. polycapillaries reach micrometer levels for the focal size. At the extreme dimensions Synchrotron radiation sources and special optics allow beam dimension as small as 50 nm. Due to the XRF method applied both qualitative (element only) and quantitative analysis is possible having at each data point the complete spectral information about the elements present in the sample and information about the elemental distribution across the analyzed area. As interesting remark by inserting a focusing element in front of the detector and align source focus and detector focus in such a way that they form a matching single point a confocal arrangement is resulting. This allows a 3-D imaging as only spectral signals from the overlapping region of the 2 focii are collected. Scanning one area followed by a second scan changing only the depth position of the focii versus the sample a 3-D image can be composed without mathematical reconstruction techniques. As a result one can expect information about the elemental distribution across interesting areas, as e.g. areas of highly metabolic activities. Results will be presented showing correlation among interesting elements in bone Pb and Zn which is found to be 10 times higher in the tidemark (the border between articular and calcified cartilage) as elsewhere in articular bone when scanning across several regions of interest. Interesting remark is that the focal size available in lab or at synchrotron sources offers new aspects to unveil in detail fine structures as can be seen in the double tide mark case which shows to be one younger and one older. In conclusion Synchrotron radiation and the suitable X-ray optics are required to create a beam of such dimension in the nm regime and still having enough photons useful for excitation of X-ray fluorescence.

References

N.Zoeger et al., OsteoArthritis and Cartilage (2006) 14, 906e913

Turyanskaya et al, 2016. μXRF Elemental Mapping of Bioresorbable Magnesium-Based Implants in Bone. Materials (Basel). 9, 811. https://doi.org/10.3390/ma9100811

Rauwolf et al., 2017. Increased zinc accumulation in mineralized osteosarcoma tissue measured by confocal synchrotron radiation micro X-ray fluorescence analysis. X-Ray Spectrom. 46, 56–62. https://doi.org/10.1002/xrs.2727

A.Gaal et al., Journal of Trace Elements in Medicine and Biology 47 (2018) 79–88

C.Streli and P.Wobrauschek, J.App. Rad and Isotop, 2019 submitted

> IL072. Invited Lecture
Symposium MED-12 Synchrotron radiation (Peter Wobrauschek)

ASSESSING THE EFFECTS OF OVARIAN CANCER TREATMENTS THROUGH SYNCHROTRON RADIATION BASED X-RAY ANALYSIS

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With more than 65.000 new cases annually in Europe, ovarian cancer (OC) represents the second most common gynecological malignancy. In comparison to other common solid cancers, OC is often diagnosed in an advanced stage, because of a lack of specific symptoms at earlier stages. Consequently, 70% of patients are diagnosed with stage IIIC OC, of which the majority are of epithelial origin. When untreated, the outlook of these patients is poor, with long-term survival (>10 years) of 10-30% for women older than 65 years.

Despite the fact that OC is usually widespread throughout the peritoneal cavity at the time of diagnosis, the disease generally remains confined to the peritoneal cavity. Therefore, intraperitoneal chemotherapy (IPC) after cytoreductive surgery has become the standard treatment in patients with peritoneal carcinomatosis of OC. By intraoperatively perfusing the abdomen of a patient with a chemotherapy solution, remaining tumor cells are directly exposed to a high concentration of cytotoxic drugs. Because of the barrier function of the peritoneal wall, systemic absorption and the toxicity thereof are limited.

Intraperitoneal chemotherapy is a technique that has proceeded quickly from bench to bedside and this left many basic questions unanswered, one of the most pressing being the penetration, diffusion and effectivity of cytotoxic drugs in tumors. The existing animal and human studies mainly look at normal tissues and general pharmacokinetics, which reveals little of the actual effect on tumors. The lack of in-depth research has led to the clinical use of dozens of untested combinations of temperature, duration, perfusate solution and drugs. There is a need for detailed research on the effect of these intraperitoneal drugs on tumors to help determine the optimal therapy.

This presentation will focus on synchrotron radiation based experiments during which several IPC protocols with cisplatin were analysed. The IPC protocols were performed on nude athymic mice with peritoneal nodules of a human ovarian cancer cell line (SKOV-3). The chief variables of these protocols were temperature, concentration and treatment duration. Nanoscopic XRF imaging was used to determine the distribution of platinum (Pt, the indicative element of the cisplatin drug) in the tumor sections, which gives information of the drug penetration under varying administration protocols. The accumulation sites of Pt give insight in the way the drug enters the tumor nodules.

This case study is a clear example demonstrating the great added value of (nanoscopic) synchrotron radiation based analysis in biomedical studies, with both direct implications for patients care as well as yielding fundamental scientific insights.

> IL073. Invited Lecture
Symposium MED-12 Synchrotron radiation (Peter Wobrauschek)

TARGETING ANTITUMOR COPPER COMPLEXES AND IMAGING USING SYNCHROTRON RADIATION AT THE CELLULAR LEVEL

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Introduction

A broad range of antitumor metal chelating compounds and metal complexes has become the focus of interest, due to their low toxicity, and their special characteristic of avoiding multidrug resistance. Copper overloading by chelators is shown to be a highly effective method for eliminating tumor cells. Since *in vivo* application of copper-based therapeutics are limited because of low solubility or fast elimination, different targeted treatments are under investigations. In the case of copper ionofores, the copper-induced cellular toxicity has a strong correlation with significant cellular copper accumulation leading to significant inhibition of cancer cell proliferation. In this study, a liposome based targeting method is presented for a copper ionofore-copper system, and cellular copper localization was determined by scanning X-ray fluorescence (XRF) imaging.

Methods and Results

Cellular level elemental imaging was made on different adenocarcinoma cell lines in the presence of Cu(II) and chelators using 8-hydroxiquinoline, phenantroline and thiosemicarbazone structures. XRF microscopy was performed at beamline B16 of the Diamond Light Source (Harwell Science and Innovation Campus, Oxfordshire, UK). A monochromatic beam of 17 keV from a multilayer monochromator was chosen to excite elements from Cl to Zn. A Kirkpatrick-Baez focusing optic was used to obtain an X-ray beam with a spot size of 650 nm × 450 nm. The XRF spectra from the specimen were acquired with a four-element energy dispersive SDD detector, while raster-scanning of the sample was performed with a step size of 500 nm × 500 nm, 5 s measuring time per point. Spectral analysis of the fluorescence spectrum of each pixel then provided images of the spatial distribution of each element. Human tumor cell lines, namely HT-29 colon adenocarcinoma, MCF-7 human breast adenocarcinoma were used. For X-ray imaging, cancer cells were grown on 7.5 mm × 7.5 mm low stress silicon nitride windows with a thickness of 500 nm. Copper treatments were performed with 2 μM copper sulfate for 1 h and 5 μM of the chelators. Images for P, S and K serve for delimitation of the cells. Considerable amounts of Cu could be localized mainly in the nuclei, however in a diffuse way. Moreover, colocalization of Cu and Zn could be observed in several cases. The extent of colocalization was investigated by Pearson correlation setting a 30% threshold compared to the maximum intensity for the fluorescent intensity data. In some cases a strong correlation (over 0.8) between the localization of Cu and Zn was found. This phenomenon may have implications in the targeting of Zn-containing peptides/proteins by the copper accumulating ionofores.

> IL074. Invited Lecture
Symposium MED-12 Synchrotron radiation (Peter Wobrauschek)

GADOLINIUM MAPPING IN BONE BY XRF SPECTROSCOPY

Authors: Anna Turyanskaya[1], Mirjam Rauwolf[1], Vanessa Pichler[1], Rolf Simon[2], Oliver J. L. Fox[3], Kawal J. S. Sawhney[3], Manfred Burghammer[4], Jochen G. Hofstaetter[5, 6], Andreas Roschger[6, 7], Paul Roschger[6], Peter Wobrauschek[1], Christina Streli[1]
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Gadolinium-based contrast media are routinely used in magnetic resonance imaging (MRI). Until recently gadolinium-based contrast media were considered to be rather safe in diagnostical applications, though in patients with kidney disease it could cause nephrogenic systemic fibrosis [1]. However, some studies revealed that gadolinium (Gd) is not cleared from the body completely and that some tissues and organs retain gadolinium for a very long time [2,3]. Free gadolinium is extremely toxic for the human organism, and, if released from depot, might pose a serious health threat.

The recent findings showed that the contrast agent is not fully excreted, and the accumulation of gadolinium in brain tissue even in subjects without renal dysfunction was observed [3,4]. The other possible depots of gadolinium are bone and cartilage tissue.

To prove this assumption, various samples of bone tissue from patients, who previously received MRI, were analyzed using 2 D imaging with 20µm and higher resolution (< 1 µm). Our aim was to investigate the distribution of gadolinium in human bone, and the obtained results will be demonstrated. To the best of our knowledge these measurements are the first attempt of imaging of Gd accumulations in the bone tissue, which is of exceptional interest for understanding of mechanisms of such accumulations and, further, for predictions of safety of Gd-based contrast media in different bone diseases and associated conditions.

References

[1] Grobner, T. Nephrol. Dial. Transplant. 2006, 21, 1745–1745.
[2] Darrah, T. H.; Prutsman-Pfeiffer, J. J.; Poreda, R. J.; Ellen Campbell, M.; Hauschka, P. V; Hannigan, R. E. Metallomics 2009, 1, 479–88.
[3] Kanda, T.; Fukusato, T.; Matsuda, M.; Toyoda, K.; Oba, H.; Kotoku, J.; Haruyama, T.; Kitajima, K.; Furui, S. Radiology 2015, 276, 228–32.
[4] McDonald, R. J.; McDonald, J. S.; Kallmes, D. F.; Jentoft, M. E.; Murray, D. L.; Thielen, K. R.; Williamson, E. E.; Eckel, L. J. Radiology 2015, 275, 772–782.

> P022. Poster

Symposium MED-12 Synchrotron radiation (Peter Wobrauschek)

ANALYSIS OF ZINC IN OSTEOSARCOMA TISSUE BY SYNCHROTRON RADIATION MICRO XRF

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Abnormal tissue levels of certain trace elements such as Zinc (Zn) were reported in various cancer types [1]. However, very little is known about the role of Zn in osteosarcoma.

Using confocal synchrotron radiation micro X-ray fluorescence analysis (SR-μXRF) at the ANKA FLUO beamline (Karlsruhe, Germany), we characterized the spatial distribution of Zn in high-grade sclerosing osteosarcoma tissue of nine patients (4 women /5 men) following chemotherapy and wide surgical resection. Zn levels in mineralized osteosarcoma tissue were compared to levels in adjacent normal healthy tissue. Quantitative backscattered electron imaging (qBEI) as well as histological examinations were also performed.

We can report the following results: on average, the ratio of medians of Zn count rates (normalized to calcium) in mineralized tumor tissue was about 6 times higher than in normal tissue. There was no difference in Zn levels between tumor fraction areas with a low and a high fraction of mineralized tissue, which were clearly depicted using qBEI [2]. Moreover, we found no correlation between the Zn values and the type of tumor regression according to the Salzer-Kuntschik grading [3].

The underlying mechanism of Zn accumulation remains unclear. Given the emerging data on the role of trace elements in other types of cancer, our novel results warrant further studies on the role of trace elements in bone cancer.

References

[1] A. Al-Ebraheem, K. Geraki, R. Leek, A. L. Harris, M. J. Farquharson, *X-Ray Spectrom.* **2013, 42, 4**.
[2] M. Rauwolf, B. Pemmer, A. Roschger, A.Turyanskaya, S. Smolek, A. Maderitsch, P. Hischenhuber, R. Simon, S. Lang, S. E. Puchner, R. Windhager, K. Klaushofer, P. Wobrauschek, P. Roschger, J. G. Hofstaetter, C. Streli, *X-Ray Spectrom.*, **2017, 46, 1.**
[3] M. Salzer-Kuntschik, G. Delling, G. Beron, R. Sigmund, *J. Cancer Res. Clin. Oncol.* **1983; 106 Suppl**.

> IL075. Invited Lecture
Symposium MED-13 Drug photosensitivity (Virginie Lhiaubet-Valle)

CLINICAL MANIFESTATIONS OF DRUG PHOTOSENSITIVITY

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Clinical manifestations of drug phototosensitivity are polymporphic and it is not always easy to distinguish phototoxicity from photoallergy, also because both mechanisms can be involved in the final reaction

Acute exaggerated sunburn or eczema of photoexposed are the main presentations of systemic photosensitivity. Pseudoporphyria, photoonycholysis, dyschromia and subacute lupus erythematosus are forms of subacute drug photosensitivity. Phototoxic drugs enhance photoaging and can also enhance photocarcinogenesis with increasing and early occurence of nonmelanoma skin cancer (or melanoma), described namely voriconazole or vemurafenib.

Main topical drugs causing photosensitivity are the NSAID, particularly ketoprofen and phenothiazine (promethazine) whereas the list of sytemic drugs causing photosensitivity is increasing everyday

Photopatch testing, indicated mainly for the study of photoallergic contact dermatitis, can also be useful in systemic drug photosensitivity, but often photoprovocation may be necessary

>IL076. Invited Lecture
Symposium MED-13 Drug photosensitivity (Virginie Lhiaubet-Valle)

CLINICAL ASPECTS OF DRUG PHOTOSENSITIVITY

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The majority of drugs used in medicine absorb light and therefore can theoretically cause photosensitivity. This may be an adverse effect, although can sometimes be used for therapeutic benefit. Most systemic drug photosensitivity reactions are non-immunological and phototoxic. Photoallergic reactions to drugs are less clearly understood and are currently most relevant for topical sunscreens and non-steroidal anti-inflammatory drugs, which are the common culprits of topical photoallergy. There are also other mechanisms for drug-induced photosensitivity, e.g. lupus, lichenoid reaction or pseudoporphyria. Investigation of drug-induced photosensitivity is undertaken in centres with photobiology expertise. The Gold Standard investigation for systemic drug phototoxicity is monochromator phototesting, which is important in distinguishing drug-induced photosensitivity from other causes of photosensitivity, as drugs usually sensitise to the UVA part of the spectrum. Monochromator phototesting is also important for photosafety investigation of new drugs with respect to defining phototoxic risk if pre-clinical signals are positive. There are common culprits for systemic drug-induced phototoxicity, such as fluoroquinolones, doxycycline, thiazides, quinine, non-steroidal anti-inflammatories and amiodarone. The investigation of choice for suspected topical photoallergy is photopatch testing and sunscreens and topical non-steroidal anti-inflammatories are the main agents implicated. Therapeutic use of drug photosensitivity is widely used in both psoralen UVA photochemotherapy and photodynamic therapy. Associations between drug-induced phototoxicity and photocarcinogenicity are not well defined, although there is clear evidence for phototoxic drugs such as psoralens, voriconazole and azathioprine.

> IL077. Invited Lecture
Symposium MED-13 Drug photosensitivity (Virginie Lhiaubet-Valle)

PHOTOCHEMISTRY OF DRUG PHOTOSENSITIZATION

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Modern lifestyle that often combines sunlight exposure with the presence of chemical substances in the skin has boosted the reports on photosensitizing effects of drugs. Over the years, numerous pharmaceuticals such as nonsteroidal anti-inflammatory agents, fluoroquinolone antibiotics or phenothiazine neuroleptics have been recognized for their photosensitizing properties.[1] The photosensitizing history of a drug encompasses clinical observation in patients, photopatch tests but also 3T3 NRU assay and, fundamental studies taking into account its photophysical and photochemical properties. In this context, a large number of efforts have been made to design a model system for photosafety assessment to establish the molecular mechanisms responsible for these side effects.

Here, an overview of this mechanistically based strategy is presented. It addresses the study of drug photophysical properties, as well as the mapping of interaction with key biomolecules (or their building blocks). This will be illustrated with our latest results dealing with the photosensitizing properties of drugs with DNA or proteins.[2]

Acknowledgments
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References

1. V. Lhiaubet-Vallet and M. A. Miranda, “Phototoxicity of Drugs” *in Handbook of Organic Photochemistry*, **2012**, Chap. 66, pp. 1541–1555. V. Lhiaubet-Vallet and M. A. Miranda, “Singlet-Oxygen Generation by Drugs and Their Metabolites” *in Singlet Oxygen: Applications in Biosciences and Nanosciences*, **2016**, Chap. 14, pp 287-303. V. Lhiaubet-Vallet, F. Bosca and M. A. Miranda *Photochem. Photobiol.* **2009**, *85*, 861–868.
2. G. Nardi, V. Lhiaubet-Vallet, and M. A. Miranda, *Chem. Res. Toxicol.* **2014**, *27*, 1990-1995. M.-D. Li, Z. Yan, R. Zhu, D. Lee Phillips, I. Aparici-Espert, V. Lhiaubet-Vallet, and M. A. Miranda, *Chem. Eur. J.* **2018**, *24*, 6654 – 6659. E. Nuin, D. Pérez-Sala, V. Lhiaubet-Vallet, I. Andreu and M. A. Miranda *Frontiers in Pharmacol.*, **2016**, *7*, 277.

> IL078. Invited Lecture
Symposium MED-13 Drug photosensitivity (Virginie Lhiaubet-Valle)

LOOKING FOR PHOTOCHEMOTHERAPEUTIC PROPERTIES OF FLUOROQUINOLONES: PHOTOTOXICITY ENHANCEMENT

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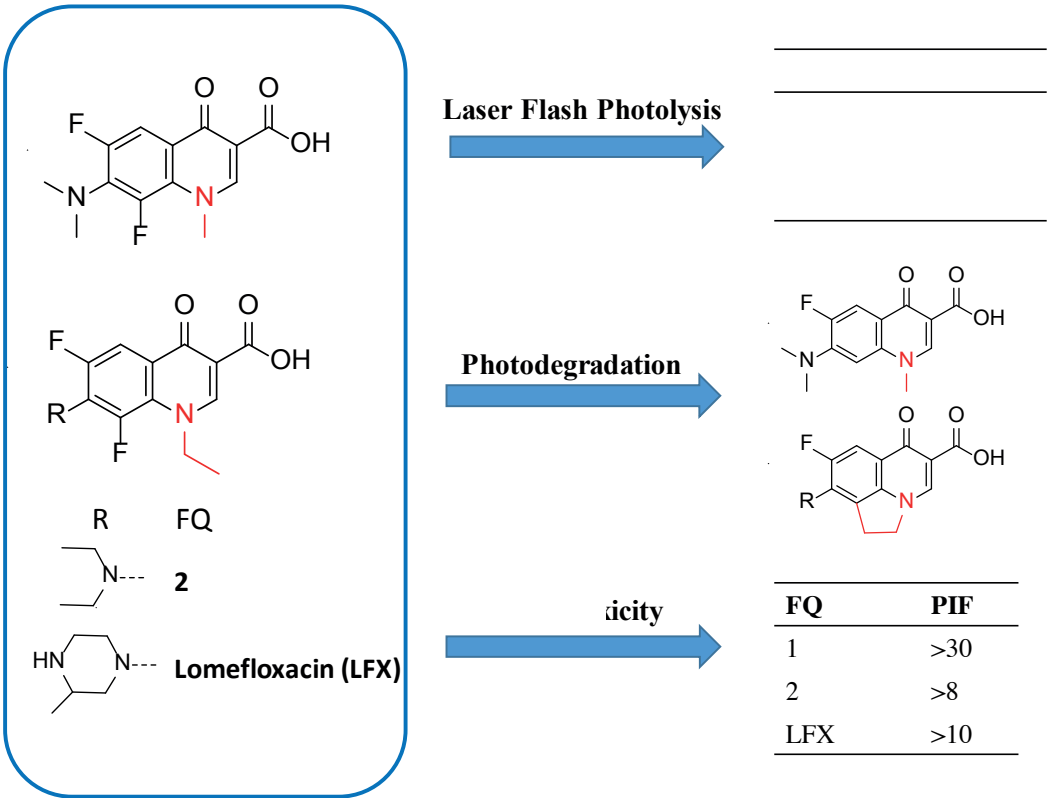
Structural modifications on quinolones have shown that this type of compounds can display antitumor and/or antiviral activities. In this context, fluoroquinolones (FQ), compounds with high activity against eukaryotic topoisomerase that exhibit relevant toxicity to cultured mammalian cells and in vivo tumor cells, could be a source of new anticancer agents.[1] Moreover, the genotoxic effects enhancement exhibited by FQ in eukaryotic systems by UV irradiation also confers to these drugs a potential property as photochemotherapeutic agent.[2] Thus, a new 1-methyl 6,8 dihalogenated quinolone **1** was synthetized looking for improving the phototoxic properties of FQ and also for determining the role of the photodegradation pathways in the FQ phototoxicity. Thereby, fluorescence emissions, laser flash photolysis experiments and photodegradation studies were performed with compound **1** using as reference compounds the 1-ethyl dihalogenatedquinolone **2** and lomefloxacin (LFX). The shortening of alkyl chain of the N(1) of the quinolone ring produces a lifetime increase of the aryl cation generated from photolysis of the three compounds and a significant reduction of the FQ photodegradation quantum yield. This difference was smaller when the same study was done using a hydrogen donor solvent, which evidenced the highest ability of the reactive intermediate arising from **1** to produce intermolecular alkylations. These results were correlated with *in vitro* 3T3 NRU phototoxicity test. Thus, when Photo-Irriation-Factor (PIF) was determined for **1**, **2** and LFX using cytotoxicity profiles of BALB/c 3T3 fibroblasts, a PIF more higher than 30 was obtained for **1** while the values for **2** and LFX were only higher than 8 and 10, respectively. Hence, the present study illustrates an approach to modulate the photosensitizing properties of FQ with the purpose to improve the chemotherapeutic properties of antitumor quinolones. Moreover, this study also evidences that the key reactive intermediate responsible for the phototoxic properties associated with dihalogenated quinolones is an aryl cation.

References

[1] Palumbo, M.; Gatto, B.; Zagotto, G.; Palu, G., On the mechanism of action of quinolone drugs. *Trends Microbiol.*, *1*, 232-5; 1993.
[2] Perrone, C. E.; Takahashi, K. C.; Williams, G. M., Inhibition of human topoisomerase IIalpha by fluoroquinolones and ultraviolet A irradiation. *Toxicol. Sci.*, *69* (1), 16-22; 2002.

Continued



FQ	PIF
1	>30
2	>8
LFX	>10

> IL079. Invited Lecture
Symposium MED-13 Drug photosensitivity (Virginie Lhiaubet-Valle)

PHOTOCLEAVAGE OF BLEBBISTATIN AS A ONE-PHOTON BLUE OR TWO-PHOTON NEAR-INFRARED LIGHT-GATED HYDROXYL RADICAL PHOTOCAGE

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Introduction

The oxygen-dependent character of conventional photodynamic therapy (PDT) makes it inadequate in certain therapy contexts such as hypoxic tumors and thus it is desirable to develop chemically tunable photocages for photoactivated chemotherapy (PACT) that do not need the presence of oxygen in the surrounding environment. PACT can be thought of as an alternative to PDT in which oxygen free reaction mechanisms can be utilized to produce cytotoxic reactive oxygen species (ROS) directly from visible light cleavable photocages.

Results and Discussion

In this talk we investigate the detailed mechanisms of the small molecule blebbistatin whereby it can be function as a one-photon blue light-gated or two-photon near-infrared light-gated photocage to directly release a hydroxyl radical (•OH) without the need for oxygen present in the surrounding environment. We utilized femtosecond transient absorption spectroscopy and chemoselective ROS fluorescent probes to study the dynamics and reaction outcomes of blebbistatin during blue light photolysis. This work revealed a water-dependent photochemistry in which a crucial process of water-assisted protonation and excited state intramolecular proton transfer (ESIPT) drives the production of short-lived intermediates that surprisingly leads to the release of •OH but not superoxide or singlet oxygen from blebbistatin. Quantum Mechanical calculations indicate that hydrogen bonding between water and blebbistatin causes this process to occur. Blue light was determined to cause blebbistatin to induce mitochondria-dependent apoptosis.

Conclusion

Our study shows blebbistatin to behave as a controllable photocage for •OH production and provides insight into the potential development of novel PACT agents.

Reference

J. Am. Chem. Soc. 2018, 140, 15957−15968

> IL080. Invited Lecture
Symposium MED-13 Drug photosensitivity (Virginie Lhiaubet-Valle)

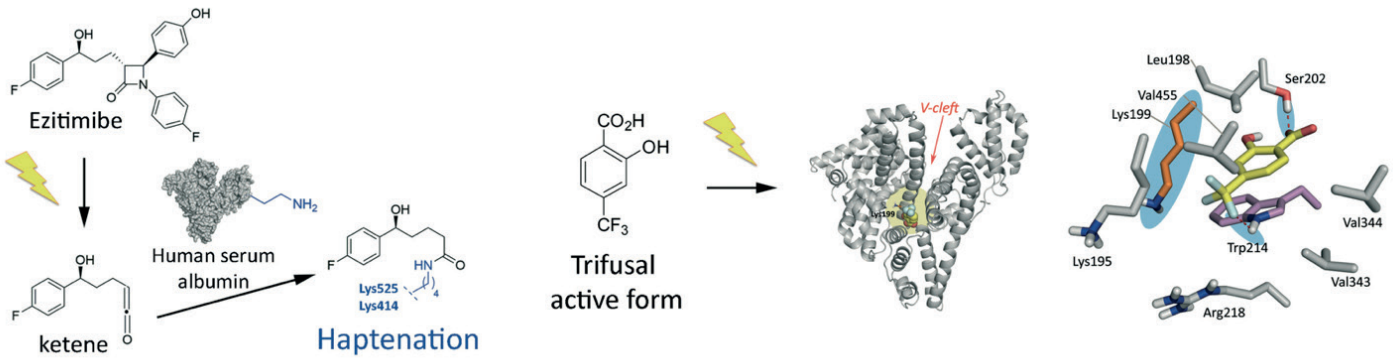
PHOTOBINDING TO HUMAN SERUM ALBUMIN BY ß-LACTAMS AND TRIFLUOROMETHYLPHENOL-CONTAINING DRUGS

Authors: Concepción González Bello[1]
Presenting Author: Concepción González Bello
1) Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), Universidade de Santiago de Compostela

A number of widely used pharmaceutical substances have been found to be associated with chemical photoallergy, which includes antibiotics, antifungals, antihistamines, cardiovascular drugs and non-steroidal anti-inflammatory drugs. Upon UV-Vis radiation, whether of solar or artificial origin, these small organic molecules can undergo bioactivation *in vivo*, which affords electrophilic species able to react with biomacromolecules, leading to covalent adducts that trigger undesired toxic effects. An integrated approach that combines photochemical, proteomic and computational studies have been used to understand in atomic detail the molecular basis of the photobinding of certain drugs to human serum albumin – the most abundant protein in plasma. The monocyclic β-lactam ezetimibe – a recently marked monocyclic β-lactam used to decrease the plasma cholesterol levels, and trifluoromethylphenol-containing drugs, such as trifusal, which is a platelet antiaggregant employed for the treatment and prevention of thromboembolic diseases were selected for these studies. Here we present a novel protein haptenation pathway by β-lactams that is alternative to the known nucleophilic ring opening of β-lactam core by the e-amino group of lysine residues. The process involves the photochemical ring splitting of the β-lactam ring to give a highly reactive ketene intermediate that is trapped by the neighbouring lysine residues, leading to an amide adduct. Moreover, the major photodegradation pathway of trifusal, which is quickly biotransformed into its active metabolite, the 2-hydroxy-4-trifluoromethylbenzoic acid, is the nucleophilic attack at the trifluoromethyl moiety by the free amino group of lysine residues to afford an amide adduct. Docking and Molecular Dynamics simulation studies provide an insight into the molecular basis of the selectivity of the two drugs for certain HSA sub-domains as well as the covalent modification mechanism. The computational studies also reveal a positive cooperative binding that explains the experimentally observed modifications in hardly accessible pockets.

References

1. D. Limones-Herrero, R. Pérez-Ruiz, E. Lence, C. González-Bello, M. A. Miranda, M. C. Jiménez, *Chem. Sci.* **2017**, *8,* 2621.
2. R. Pérez-Ruiz, E. Lence, I. Andreu, D. Limones-Herrero, C. González-Bello, M. A. Miranda, M. C. Jiménez, *Chem., Eur. J.* **2017**, *23,* 13986.
3. O. Molins-Molina, E. Lence, D. Limones-Herrero, C. González-Bello, M. A. Miranda, M. C. Jiménez. *Org. Chem. Front.* **2019**, *6*, 99.
4. O. Molins-Molina, E. Lence, C. González-Bello, M. A. Miranda, M. C. Jiménez, *J. Org. Chem.* **2018**, *83*, 13019.
5. O. Molins-Molina, R. Pérez-Ruiz, E. Lence, C. González-Bello, M. A. Miranda, M. C. Jiménez, submitted.



> IL081. Invited Lecture
Symposium MED-13 Drug photosensitivity (Virginie Lhiaubet-Valle)

KNOWLEDGE IS POWER: UNDERSTANDING THE ACTION OF PHOTODRUGS AND THEIR SIDE EFFECTS BY MOLECULAR MODELING AND SIMULATION

Authors: Antonio Monari[1]
Presenting Author: Antonio Monari
1) LPCT Université de Lorraine and CNRS

The secondary effects of photodrugs and photosensitivity are usually related to a complex cascade of molecular events linked together by complex cross-talk happening in complex and crowded enviropnments. Hence, the proper and ultimate rationalization of all the processes into play, and obviously of the biological outcome is, in many case complicated.

Recently molecular modeling and simulation, using both quantum chemistry and classical molecular dynamics, has allowed gaining an unprecedented insight into the behavior of complex chemical and biological processes with an atomistic or even electronic resolution. Leading to what is usually referred as the emergence of a computational microscope.

We will illustrate, by a series of chosen examples, all the information that molecular modeling and simulations may provide in determining the molecular basis of the action of different photodrugs in term of their interaction with biological macromolecules, such as nucleic acid or biological membranes, and the induced modification in their photophysical or photochemical behavior. The crucial aspects of drug delivering and the exploitation of photophysical and photochemical phenomena in favoring drug uptake and reducing their size effects will allso be tackled. Finally and as an example of the possibilities offered by lolecular modleing the elucidation of a secondary effect of a phototherapeutic drug inducing vision hypersensibilization (night vision) will be presented.

Through this talk we aim at clearly showing how molecular modeling and simulation may help in rationalize the mechanism of action of photo active drugs and hence understand and prevent eventual side effects.

References

1. Sengul et al. (2018) *J. Phys. Chem. C 122,* 16315
2. Yakavets et al. (2018) *J. Photochem. Photobiol. A 367,* 13
3. Francés-Monneris et al. (2018) *Phys. Chem. Chem. Phys. 20*, 25666
4. Marazzi et al. (2017) *J. Phys. Chem. B 121*, 7586
5. Gattuso et al. (2016) *Nature Sci. Rep. 6,* 28480

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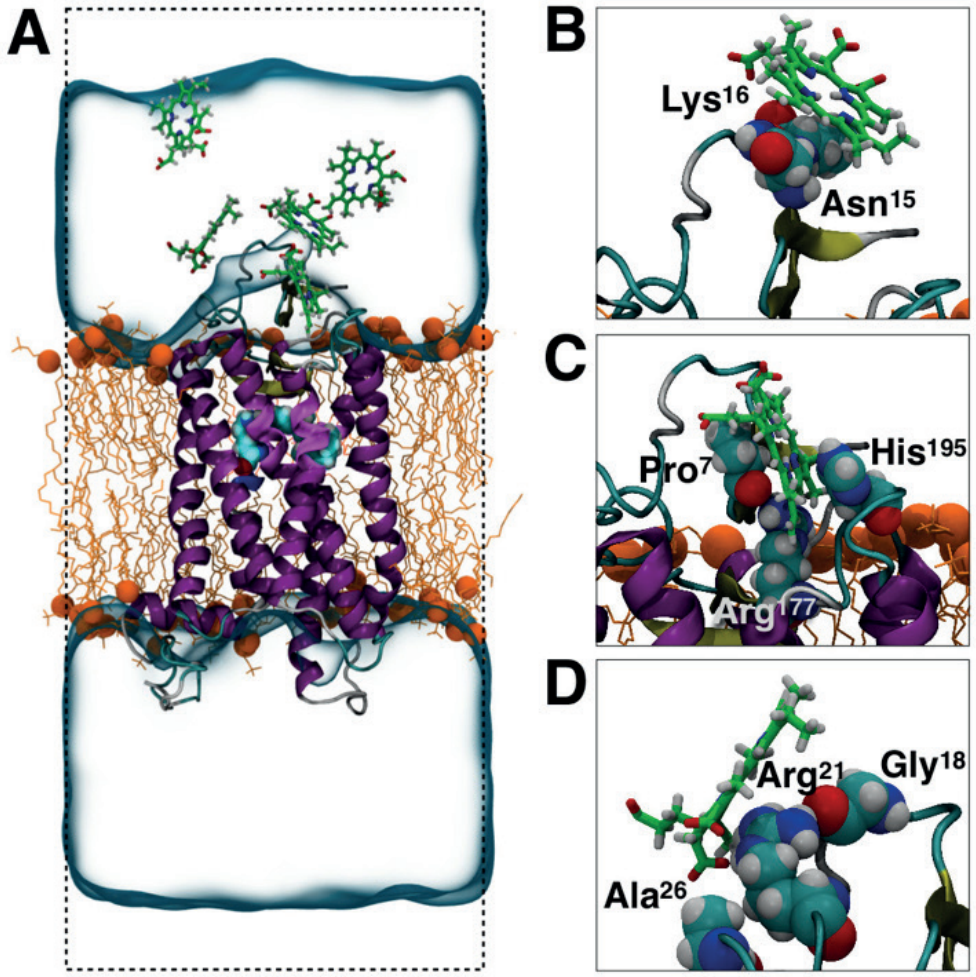
A
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Lys16
Asn15
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Pro7
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Arg177
D
Arg21
Gly18
Ala26

> OC019. Oral Communication
Symposium MED-13 Drug photosensitivity (Virginie Lhiaubet-Valle)

INVESTIGATING PIRFENIDONE-INDUCED PHOTOTOXICITY: A CRITICAL DRUG FOR IDIOPATHIC PULMONARY FIBROSIS

Authors: Alessia Baseggio Conrado[1,2], Daniel Tan[2], Jean Yu Choi[2], Sally Ibbotson[1,2], Victoria A. McGuire[1,2]
Presenting Author: Victoria A. McGuire
1) Photobiology Unit, Ninewells Hospital and Medical School, Dundee, UK 2) School of Medicine, University of Dundee, Dundee UK

Introduction
Idiopathic Pulmonary Fibrosis (IPF) is a persistent and progressive lung disease, which is extremely difficult to treat and has a high mortality rate (1). Pirfenidone is one of very few drugs that can slow disease progression and improve survival rates in patients with IPF (2), and it has been reported to have both anti-inflammatory and anti-fibrotic effects that may contribute to its efficacy. However, major side-effects include gastro-intestinal disturbances, skin rashes and photosensitivity which may cause patients to stop treatment.
The absorption of pirfenidone peaks at 315nm (UVB) and extends to 360 nm (UVA)(3). Pirfenidone-induced phototoxicity in patients with IPF has been investigated in patients referred to the Photobiology Unit and abnormal erythemal responses to ultraviolet radiation (predominantly UVA) on monochromator phototesting have been observed, confirming abnormal photosensitivity in humans, although the mechanisms by which this occurs are not well established.
This project aims to explore how pirfenidone causes abnormal photosensitivity by examining its phototoxicity in cultured skin cells. We investigated whether pirfenidone affects cell viability and assessed which wavelength(s) of light mediate these effects. Having a better understanding of how photosensitivity is caused may improve current approaches in prevention and management of symptoms and improve quality of life for patients taking this important drug.

Methods
Cultured human HaCaT keratinocytes were incubated with increasing concentrations of pirfenidone followed by UVA (5-10 J/cm^2) or UVB (15-30mJ/cm^2) irradiation. Cell viability was subsequently determined using MTT or Neutral Red uptake assays

Results and Discussion
Pirfenidone induces dose-dependent phototoxicity in HaCaT cells in response to UVA irradiation. We have so far been unable to detect pirfenidone-induced phototoxicity in response to UVB irradiation. These results are consistent with data showing that pirfenidone can generate reactive oxygen species following exposure to simulated sunlight *in vitro* (3). The mechanism of pirfenidone-induced phototoxicity is being explored further by examining free radical production in response to UVA irradiation. We also plan to investigate the effects of pirfenidone on the activation of intracellular signalling pathways leading to cytokine production in order to assess the potential influence of the pirfenidone-light interaction on the inflammation underlying IPF.

Conclusion
Pirfenidone causes phototoxicity in cultured HaCaT keratinocytes following UVA irradiation, although the mechanisms underlying this remains to be determined.

Acknowledgements
This work is supported by the British Skin Foundation and the British Lung Foundation/Cystic Fibrosis Trust

Conflicts of interest
None

References

(1) Raghu G et al. Am J Respir Crit Care Med 2011 183:788-824 (2) Noble PW et al. Eur Respir J 2016 47:243-253 (3) Seto et al. J Photochem Photobiol B 2013 120:44-51

> OC020. Oral Communication

Symposium MED-13 Drug photosensitivity (Virginie Lhiaubet-Valle)

DESIGN OF A NEW SUNSCREEN-BASED PHOTOCAGED SYSTEM. A MECHANISTIC STUDY BASED ON A MODEL AND ITS APPLICATION IN A KETOPROFEN-OXYBENZONE DYAD.

Authors: Mauricio Lineros Rosa[1], Virginie Lhiaubet-Vallet[1], Miguel A. Miranda[1]
Presenting Author: Mauricio Lineros Rosa
1) Instituto Universitario Mixto de Tecnología Química (UPV-CSIC), Universitat Politècnica de València

This work focuses the attention on the development of a new photoprotection strategy in order to counteract the photosensitizing effects of some drugs on biomolecules[1]. Among the photosensitizing substances, nonsteroidal anti-inflammatory drugs (NSAIDs) for topical use are particularly important due to their extensive use in daily life. The most representative example of this family is ketoprofen (KP), a drug that is responsible for pronounced cutaneous photosensitization[2]. That is the reason why ketoprofen has been chosen for the present work.

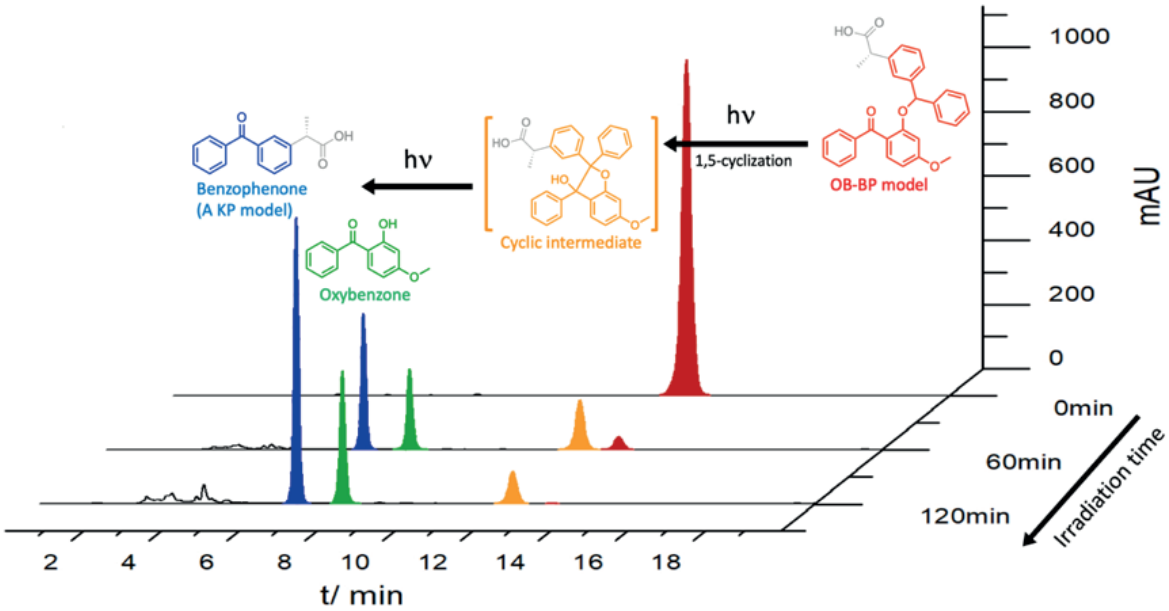
In this research, we have developed a new system able to photorelease both KP and a solar filter, oxybenzone (OB), which should prevent the harmful drug adverse effects caused by UVB and UVA radiation.

As a first step, since ketoprofen is a benzophenone derivative, a model oxybenzone-benzophenone (OB-BP) system was prepared to evaluate and optimize the photoreleaseconditions. The HPLC analysis showed that photorelease of both components (BP and OB) comes from a cyclic intermediate (OB-BP-C), which arises from an intramolecular H-abstraction and biradical recombination. Further studies demonstrated that the process takes place faster in the presence of 4-benzoylbenzoic acid (4-CBP), acting as a photooxidant. On the other hand, laser flash photolysis (LFP) experiments revealed that quenching of 4-CBP triplet excited state by OB-BP-C leads to ketyl radical formation with a quenching rate constant value of *ca.* $10^9 M^{-1}s^{-1}$. This result points toward a mechanism where generation of OB-BP-C radical cation plays an important role in the ring cleavage process in the origin of the OB and BP delivery.

Finally, as a real application of the model, the dyad oxybenzone-ketoprofen (OB-KP) was prepared. Photorelease experiments were carried out using the optimized conditions and they were assessed by HPLC. From these studies, it was observed that ketoprofen and oxybenzone are effectively photoreleased.

References

[1]I. Aparici-Espert, M. C. Cuquerella, C. Paris, V. Lhiaubet-Vallet and M. A. Miranda, *Chem. Commun.*, **2016**, *52*, 14215-14218.
[2]V. Lhiaubet-Vallet and M. A. Miranda, CRC Handbook of Organic Photochemistry and Photobiology, CRC Press, Boca Raton, 3rd edn, **2012**, ch. 66, vol. 2, p. 1541.



> P023. Poster

Symposium MED-13 Drug photosensitivity (Virginie Lhiaubet-Valle)

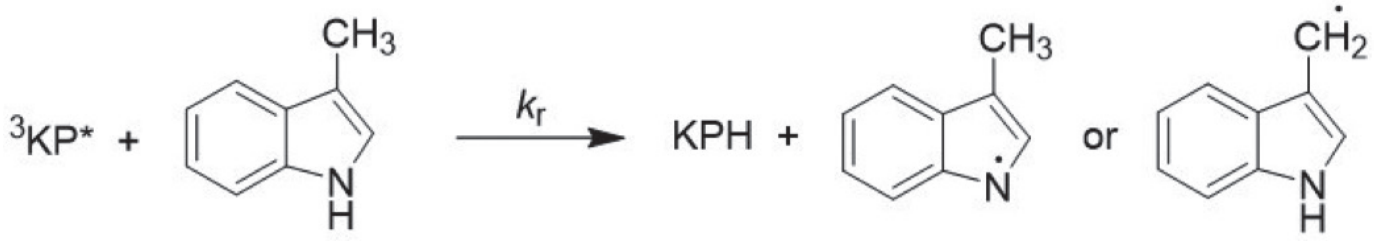
PHOTOCHEMISTRY OF KETOPROFEN WITH INDOLES

Authors: Wataru Kashihara[1], Tadashi Suzuki[1]
Presenting Author: Wataru Kashihara
1) Aoyama Gakuin University

Ketoprofen (KP) is one of the most popular nonsteroidal anti−inflammatory drugs (NSAIDs), however, photosensitization of KP has been reported in these decades [1]. To elucidate the photosensitization mechanism of KP under UV irradiation, photochemistry of KP with indoles which have a side chain of tryptophan was studied by transient absorption spectroscopy. From the precise analysis of the transient spectra it was found that KP in the excited triplet state, ^{3}KP*, abstracted a hydrogen atom from indoles to afford a ketyl radical and a counter radical. The bimolecular quenching rate constants of ^{3}KP* by indoles, k_q, and the hydrogen atom abstraction rate constants, k_r, were obtained. The values of k_r for methylindoles were larger than that of indole, revealing that ^{3}KP* would abstract a hydrogen atom of the methyl group as well as that of N–H in the indole frame. These findings will give us information on the reactivity of excited KP in the vicinity of tryptophan in a KP−protein complex.

References

[1] T. Artuso, J. Bernadou, B. Meunier, J. Piette, N. Paillous, *Photochem. Photobiol*. **1991**, *54*, 205−213.



> IL082. Invited Lecture
Symposium MED-14 Clinical Photosafety (Douglas B. Learn)

PRECLINICAL PHOTOSAFETY EVALUATION: THE TRIGGER FOR CLINICAL PHOTOSAFETY EVALUATION

Authors: Douglas Learn[1]
Presenting Author: Douglas Learn
1) Charles River Laboratories Safety Assessment, Horsham

The evaluation of photosafety for pharmaceuticals and consumer care products is governed by good ethical practice to ensure patient or user safety and good scientific design and data interpretation. The recommended approach is to perform preclinical photosafety studies to either eliminate any risk or define potential human phototoxicity risk based on the intended. These studies are outlined in the relevant international guidances, including the OECD 432 Guideline *In* Vitro 3T3 NRU Phototoxicity Test, the ICH S10 Photosafety Evaluation of Pharmaceuticals, the ICH S9 Nonclinical Evaluation for Anticancer Pharmaceuticals and the ICH M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. These guidances define the circumstances under which evaluation is recommended, the stepwise approach to these evaluations and general information on relevant study design. In nearly all instances, these studies are performed under Good Laboratory Practices regulations. This presentation will provide a review of the relevant guidances and regulations, a brief overview of the history of preclinical photosafety evaluation, a practical overview of these studies, the technical aspects employed, interpretation of the results and how these results may or do not lead to clinical photosafety evaluations.

> IL083. Invited Lecture

Symposium MED-14 Clinical Photosafety (Douglas B. Learn)

PHOTOSAFETY EVALUATION OF FRAGRANCE MATERIALS: A TIERED APPROACH USING BOTH ALTERNATIVE METHODS AND CONFIRMATORY TESTING IN HUMANS

Authors: Gretchen Ritacco[Resea]
Presenting Author: Gretchen Ritacco
1) Research Institute for Fragrance Materials, Inc. (RIFM)

The Research Institute for Fragrance Materials (RIFM), founded in 1966, is a non-profit scientific organization that supports the global fragrance industry's safe use of fragrance materials. RIFM maintains the world's most comprehensive database of toxicology data, literature, and general information on fragrance materials. RIFM has an extensive program of testing and evaluating fragrance raw materials, and this program is reviewed by an independent Expert Panel for Fragrance Safety. Additionally, RIFM's safety assessments of fragrance materials are published in peer reviewed scientific journals. Phototoxicity is one of 7 endpoints covered in our fragrance material safety assessments. This presentation will focus on the tiered approach used to assess photosafety of fragrance materials. The foundation of our testing strategy is UV/Vis absorbance spectra (OECD 101). Materials with significant UV/Vis absorbance (molar extinction coefficient > 1000 $L \cdot mol^{-1} \cdot cm^{-1}$) are considered to have the potential to cause phototoxic effects (Henry et al., 2009). We have obtained UV absorbance spectra for nearly 2000 fragrance materials and approximately 93% did not demonstrate significant absorbance. For those materials that demonstrate significant absorbance, further testing is required. Our photosafety testing is conducted in a tiered manner, moving from the hazard-based 3T3-neutral red uptake phototoxicity test (OECD 432) to the risk-based reconstructed human epidermis phototoxicity test, with no-effect levels confirmed in human phototoxicity tests. Since 2014, 101 materials have been tested in the 3T3-neutral red uptake phototoxicity assay. Eighteen of these materials were predicted to be phototoxic in the assay, and most were subsequently tested in the reconstructed human epidermis model at three concentrations. Provided the results were negative (i.e., "not phototoxic") the same three concentrations were used in a confirmatory human phototoxicity study. With this approach, a no-effect level for phototoxicity in humans is determined. Case studies of specific fragrance materials will also be presented to illustrate our approach to photosafety testing.

> IL084. Invited Lecture
Symposium MED-14 Clinical Photosafety (Douglas B. Learn)

THE CLINICAL EVALUATION OF DRUG PHOTOTOXICITY

Authors: Sally Ibbotson[1]
Presenting Author: Sally Ibbotson
1) University of Dundee

Photoactive drugs absorbing between 290 – 700 nm may theoretically be associated with phototoxic potential in the clinical setting. Photosafety evaluation of any new drug under development is required for compounds with this absorption profile and if *in vitro* and pre-clinical cell and animal phototoxic testing show positive signals for phototoxicity, then judgement is required as to whether testing in the human setting is necessary. Human volunteer testing is ideally undertaken at a phase in drug development when detailed understanding of the bioavailability and pharmacokinetics of the drug are established, and prior to it being used in large numbers of patients in later clinical trials. With respect to meeting regulatory requirements, a robust randomised clinical trial using positive and negative controls is optimal. Use of monochromator phototesting to establish minimal erythema doses (MED) as end points will enable the phototoxic index of the drug at specific wavebands across the solar spectrum to be established. In addition, the time to resolution of photosensitivity for drugs shown to be phototoxic can also be investigated. Broad-spectrum solar simulator phototesting may also be employed, although with this UVB-weighted spectrum, there is the potential to miss significant UVA photosensitivity and narrower waveband testing may be preferable in this regard. For specific drugs, the phototoxic risk should also take into account the importance of indications for the drug and alternative treatment options and whether the drug will be used acutely or long term, in addition to the nature of the patient population, for example, immunocompetent or immunosuppressed. Information regarding human volunteer testing of drugs with phototoxic risk will be discussed, in particular using fluoroquinolones as an example.

> IL085. Invited Lecture
Symposium MED-14 Clinical Photosafety (Douglas B. Learn)

METHODOLOGY TO EVALUATE THE PHOTOSENSITIVITY POTENTIAL OF AN INVESTIGATIONAL PRODUCT IN HEALTHY VOLUNTEER SUBJECTS

Authors: Jonathan Dosik[1], John Lyssikatos[1], Allyson Marshall[1], Michael Tuley[1]
Presenting Author: Jonathan Dosik
1) TKL Research Inc.

TKL Research Inc. (TKL) designed and implemented a Phase 1 clinical trial to assess the photosafety of a systemically administered investigational product (IP) using a partially-blind, randomized, parallel group, placebo-controlled study design. Healthy volunteers were enrolled and randomized in a 3:1 manner to receive the IP or placebo (Part A) or the known photosensitizing agent ciprofloxacin (Part B). Subjects in Parts A and B received the drug (IP, placebo, or ciprofloxacin) for a predetermined period followed by photosensitivity assessments for 72 hours after the administration of the last dose. Photosensitivity was evaluated by determining the minimal erythema dose (MED) testing for skin exposed to a series of ultraviolet light A and B (UVA and UVB) exposures. Skin test sites were analyzed for erythema and superficial skin reactions.

A photosensitivity study conducted with this design will yield the following results for the IP, positive control, and placebo at predetermined timepoints: $MED_{baseline}$ and $MED_{on\ drug}$, calculation of photosensitivity index (PI) following UVB/UVA and UVA-only radiation, skin grading for local skin reactions, and pharmacokinetics of IP (blood samples may be obtained to monitor pharmacokinetic parameters to ensure the IP has achieved steady state levels. Additionally, safety and tolerability were assessed via monitoring of adverse events (AEs). We propose this design as a new standard for photosensitivity clinical trials.

> IL086. Invited Lecture
Symposium MED-14 Clinical Photosafety (Douglas B. Learn)

PHOTOSAFETY EVALUATION OF PHARMACEUTICALS - USING SAFETY MARGINS TO SUPPORT HUMAN RISK ASSESSMENT

Authors: Daniel Bauer[1]
Presenting Author: Daniel Bauer
1) Novartis, Preclinical Safety, 4002 Basel, Switzerland

Phototoxic properties of systemically applied pharmaceuticals may be the cause of serious adverse drug reactions. Despite being clinically manageable in principle, they can limit the use of a drug depending on the indication. Protective measures against sunlight can be applied very reasonably during a few days but may not be practicable for chronic treatments. Thus, both patients and health authorities are unlikely to accept a relevant photosensitization risk in such situations.

Typically, a reliable preclinical photosafety assessment strategy combining *in vitro* and *in vivo* approaches is usually applied early on. For most drug candidates, photosafety evaluation can be based purely on spectroscopic measurements and *in vitro* results (Bauer, Regul Toxicol Pharmacol, 2014). However, a few compounds will need confirmation *in vivo*. Such studies involve multiple dose levels covering the pharmacologically efficacious dose and the maximal tolerated dose with the aim to define the no-observed adverse events level (NOAEL) and to derive critical PK parameters (i.e. $\boldsymbol{t}_{max}$, $\boldsymbol{C}_{max}$).

The results from 41 acute, oral murine photo-local lymph node assays (photo-LLNA) demonstrated the utility of this approach (Schümann, Toxicol Sci, 2014). Phototoxicity *in vivo* is clearly a dose-dependent effect. The applied level of simulated sunlight (normalized to 10 J/cm^2 UVA) is sufficient to elicit phototoxic responses using reference compounds and corresponds well to typical sunlight exposure of patients. Therefore, NOAEL-derived safety margins versus therapeutically relevant drug levels based on C_{max} are an appropriate method to support human risk assessment and are regulatory accepted (ICH S10, 2013).

Historically, in particular antibiotics such as fluoroquinolones or tetracyclines were extensively evaluated regarding clinically relevant photosensitivity reactions. However, during the last 20 years the majority of pharmaceutical companies have introduced preclinical photosafety testing strategies in order to avoid late stage surprises. Therefore, clinical photosensitivity testing of investigational drugs is not performed on a routine basis and only close to clinical phase 3 development trials due to the required exposure levels. The outcome of such studies is not published systematically. However, two recent cases demonstrated the relevance and utility of safety margins based on preclinical data. Vemurafenib was found clinically phototoxic at therapeutic exposure levels. *In vitro* and *in vivo* results were suggestive of this outcome (Boudon, Toxicol Sci, 2014). In contrast, Pradigastat did not demonstrate any phototoxicity in a dedicated clinical photosensitivity study (Bauer, Photochem Photobiol Sci, 2016) which confirmed the preclinical assessment indicating a margin of at least 15-fold based on the NOAEL *in vivo*.

> OC021. Oral Communication
Symposium MED-15 Short Communications on Skin Photobiology (Yolanda Gilaberte)

HOW TO PROTECT SKIN FROM VISIBLE LIGHT: MODELS AND METHODS OF PROTECTION

Authors: Eduardo Ruvolo[Bayer]
Presenting Author: Eduardo Ruvolo
1) Bayer Healthcare LLC

In the past few years, more attention has being given to the effects of visible light (VL: 400-700 nm). Visible light (VL) has been reported to induce both transient pigment as well as long lasting pigmentation induction on human skin. Pigmentation induced by VL may persist up to 8 weeks and the amount of pigment produced is dependent on the total dose of light. In addition, VL can induce significant reactive oxygen species (ROS) production, and this ROS can be inhibited, showed in several *in vitro* and *ex-vivo studies*, by the addition of anti-oxidants combo in cosmetic formulated sunscreens.

Despite the use of very effective sunscreens against UV radiation, many patients with melasma and PIH have relapses of the hyperpigmented lesions after the summer period. It is also unknown how effective are, *in vivo* study, anti-oxidants and quenchers in providing clinically relevant protection in the visible part of sun spectrum.

In this work, we will present an *in vitro* model to predict the protective effectiveness of pigments that absorb the visible part of the spectrum when applied topically on skin. This model is analogous to the *in vitro* SPF. The model utilizes a proposed IPD action spectrum in the visible portion of the spectrum and the irradiance of a visible light source used in the clinical studies to derive the protective index for visible light protection. Based on the protective *in vitro* index for visible light we will be presenting two approaches to assessing visible light protection using a topical product containing absorptive pigments. One method determines a protection index – similar to an SPF value, for visible light only using a pigmentation endpoint. A second method uses multiple exposures and measures the magnitude of the suppression by the protective topical formulation compared to an unprotected area simulating daily exposure.

To understand how effective anti-oxidants and quenchers can effectively suppress the effects of ROS induced by VL+UVA1 on human skin, the results from a clinical study using an antioxidant/quencher complex will be presented.

> OC022. Oral Communication
Symposium MED-15 Short Communications on Skin Photobiology (Yolanda Gilaberte)

THE DUTCH SOLAR INTENSITY ACTION PLAN

Authors: Arjan van Dijk[1], Werner Hagens, Mariska Boekema
Presenting Author: Arjan van Dijk
1) RIVM (National Institute for Public Health and the Environment), The Netherlands

Introduction

Harmful effects from UV-exposure are manifest. Skin cancer incidence in the Netherlands has gone up by a factor of 4 in the past 25 years and another factor of 5 increase is foreseen in the coming 25 years. Many different organizations in the Netherlands engage in skin cancer prevention, but their actions are incoherent. Consequently, the public is left a divided: “What to do?”. The government has called for action and started a project to come to a unified approach, called the “Solar Intensity Action Plan”.

Methods

A consortium of all stakeholders is formed: ministry of health, national institute of public health and the environment, national weather service, society of dermatologists, society of eye doctors, cancer prevention foundation, skin care foundation, eye care foundation, several academic hospitals, the cancer registry bureau and the society of melanoma patients. Commercial parties are excluded from participation until further notice, but individual partners are allowed to have professional relations with them. The goals of the (growing) consortium are: coordination of communication strategies, exchange of experience, maintenance of a common knowledge agenda (including best practices from other countries), prioritization of knowledge gaps and compilation of scientific research proposals to address these gaps. A web-based discussion forum is used to facilitate and organize discussions among the members on the relevant topics.

Results and Discussion

The project is in its initial stage. An inventory is being made of the penetration of all partners in the different target groups in society, of the respective advices that are given and of the communication strategies to convey them. An agenda is compiled for national or regional regular communications in the name of the consortium, e.g. when a high UV-index is expected, at the start of the (summer and winter) holiday season, national holidays, large festivals etc. We seek a way to assess today the effectiveness that the Solar Intensity Action Plan will have in the future. Due to lag of several decades between exposure and resulting skin cancer, we cannot afford to monitor just the development of the skin cancer incidences. In a few decades, dramatically risen incidences will be a fait accompli. We must take the right action now.

Conclusions

UV-exposure has to change to avert a disaster and the Solar Intensity Action Plan is the Dutch initiative to reach this goal. Suggestions are welcome.

> OC023. Oral Communication

Symposium MED-15 Short Communications on Skin Photobiology (Yolanda Gilaberte)

IDENTIFICATION OF TWO INDEPENDENT UVB-INDUCED CELL DEATH PATHWAYS IN HUMAN DERMAL FIBROBLASTS

Authors: Anne-Sophie Gary[1,2], Patrick J. Rochette[1,2,3]
Presenting Author: Anne-Sophie Gary
1) Axe Médecine Régénératrice, Centre de recherche du CHU de Québec – Université Laval, Hôpital du Saint-Sacrement (Laval University, Quebec, Canada). 2) Centre de recherche en organogénèse expérimentale de l'Université Laval / LOEX (Tissue Engineering Laboratory / LOEX). 3) Département d'Ophtalmologie et ORL - chirurgie cervico-faciale, Faculté de médecine, Université Laval.

Ultraviolet B (UVB) radiation is the main responsible for non-melanoma skin cancer induction. By eliminating the most damaged cells, programmed cell death, such as apoptosis, is considered a protective mechanism against cellular transformation. Apoptosis is characterised by the activation of caspases and is known to be activated by UVB radiation. UVB exposure induces DNA damage, oxidation and death receptor activation, all leading to apoptosis [1]. In addition to UVB-induced apoptosis at 16-24h post irradiation of fibroblasts, we have observed a necrotic-like population 3h-6h post-UVB. In this project, we aim to determine the different UVB-induced cell death in dermal fibroblasts. We have thus assessed the activation of different cell death (necroptosis, ferroptosis, apoptosis and PARP-dependant cell death) post-UVB, along with their mechanism.

Methods

Primary cultures of human dermal fibroblasts were irradiated with a lethal UVB dose (20 or 30 kJ/m^2). Using different inhibitors of necroptosis, ferroptosis, apoptosis and PARP, we have determined the contribution of each pathways in UVB-induced cell death. Cell viability was assessed using MTS assay at 0, 1, 3, 6 and 24h post-UVB.

Results

As predicted, we observed UVB-induced apoptosis. Interestingly, we also identified a caspase-independent PARP-dependent cell death which occurs earlier than the apoptosis. Indeed, PARP-dependant cell death is activated between 3 to 6h post-UVB, while caspase-dependent death is observed at 24h. The combination of caspase and PARP inhibitors abolished virtually all cell death post-UVB, indicating that both cell death act independently.

Discussion

Our results provide evidence that UVB-induced cell death in fibroblasts take place in two different sequential events, i.e. an early PARP-dependant cell death and a late apoptosis cell death. We hypothesize that the PARP-dependent cell death is in fact parthanatos and we are currently performing experiments investigating AIF translocation to confirm it. Indeed, parthanatos is characterised not only by the involvement of PARP, but also by the translocation of AIF from mitochondria to nucleus [2]. To our knowledge, it is the first evidence of the involvement of 2 independent cell death pathway following UVB irradiation in skin cells.

Conflicts of interests

There is no conflict of interest.

References

[1] D. Kulms and T. Schwarz, "Independent contribution of three different pathways to ultraviolet-B-induced apoptosis," *Biochem. Pharmacol.*, vol. 64, no. 5–6, pp. 837–841, 2002.
[2] Y. Wang, V. L. Dawson, and T. M. Dawson, "Poly(ADP-ribose) Signals to Mitochondrial AIF: A Key Event in Parthanatos," *Exp Neurol*, vol. 218, no. 2, pp. 41–43, 2010.

> OC024. Oral Communication

Symposium MED-15 Short Communications on Skin Photobiology (Yolanda Gilaberte)

HARNESSING ULTRAVIOLET LIGHT TO REDUCE METABOLIC DYSFUNCTION THROUGH NITRIC OXIDE

Authors: Sian Geldenhuys[1], Naomi Fleury[1], Gursimran Dhumrait[1], Tristan Allemann[1], Kunjal Panchal[1], Martin Feelisch[2], Prue Hart[1], Robyn Lucas[3], Peter Mark[4], Richard Weller[5], Vance Matthews[6], Shelley Gorman[1]
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Introduction

Sunlight and ultraviolet radiation (UVR), are essential for life and have shaped the way energy is acquired. Indeed, humans have evolved under the influence of sunlight. However, our lifestyles have dramatically changed in recent times, with more sedentary time indoors and increased consumption of energy-dense food and drink.

Methods & Results

In our pre-clinical studies, we observed that regular skin exposure to low (non-burning) doses of UVR reduced weight gain and signs of metabolic dysfunction in mice fed a high fat diet.[1] The effects of UVR were independent of circulating 25-hydroxyvitamin D and not mimicked by vitamin D supplementation. Instead, release of nitric oxide bioactivity from irradiated skin was responsible for some of the suppressive effects of UVR. Weight gain and hepatic steatosis were reduced when already 'overweight' mice (with signs of glucose intolerance) were exposed to low dose UVR, through nitric oxide.[2] Low dose UVR also had anti-inflammatory effects, with reduced liver *Tnf* mRNA levels observed.[3] We hypothesized that low dose UVR could modulate metabolism by enhancing thermogenesis (heat production) by (interscapular) brown adipose tissue (iBAT) located beneath the irradiated skin site. BAT is characterised by high levels of a marker for thermogenesis, uncoupling protein-1 (UCP-1), as observed in the UCP-1 luciferase transgenic mouse housed in cold conditions, with UCP-1 expression tracked via a bioluminescent tag.[4] Through our detailed circadian analyses, no substantial shifts in UCP-1 expression in iBAT of UCP-1 luciferase transgenic mice exposed to low dose UVR (fed a high fat diet) were identified. However, skin temperature at the interscapular skin site, and the extent of 'whitening' (white adipose accumulation) in BAT were suppressed by exposure to UVR through a nitric oxide-dependent mechanism.

Discussion & Conclusions

Low dose UVR suppressed the 'whitening', steatotic and pro-diabetic effects of consuming a high fat diet in mice fed a high fat diet, through skin release of nitric oxide, via a mechanism likely to be independent of diet-induced (UCP-1-mediated) thermogenesis in BAT. Further studies examining the effects of UVR on glucose metabolism, lipid accumulation and inflammation in metabolically active tissues, and its capacity to regulate the vascular tone and temperature of the dermis are needed. Combined with some observations of metabolic benefit from human clinical and epidemiological studies, increased exposure to sun or UV light has the potential to curb the development of type-2 diabetes and obesity.[5] However, further research needed to determine whether our pre-clinical observations are reproduced in people.

References:

1. Geldenhuys et al *Diabetes* 2014;63:3759.
2. Fleury et al *J Endocrinol* 2017;233:81.
3. Teng et al *BMC Res Notes* 2019;12:78.
4. Galmozzi et al *Cell Rep* 2014;9:1584.
5. Gorman et al *Photochem Photobiol Sci* 2017;16:362.

> OC025. Oral Communication

Symposium MED-15 Short Communications on Skin Photobiology (Yolanda Gilaberte)

EFFICACY EVALUATION OF AN ANTIOXIDANT COMPLEX ON VISIBLE LIGHT-INDUCED BIOLOGIC EFFECTS

Authors: Indermeet Kohli[1], Alexis Lyons[1], Raheel Zubair[1], Amanda Nahhas[1], Taylor Braunberger[1], Mohsen Mokhtari[2], Eduardo Ruvolo[3], Henry W. Lim[1], Iltefat Hamzavi[1]
Presenting Author: Henry Lim / Indermeet Kohli
1) Henry Ford Hospital, Detroit, MI, USA 2) Wayne State University, Detroit, MI, USA 3) Bayer Consumer HealthCare LLC., Whippany, NJ, USA

Introduction

Visible light and long wavelength ultraviolet A1 (VL+UVA1, 370-700 nm) have synergistic effects on pigmentation and erythema in darker skin individuals. This study evaluated skin responses of lighter skin individuals to VL+UVA1 which have not been evaluated previously. Efficacy of an antioxidant complex on the VL+UVA1 induced effects was also investigated for all skin phototypes (SPT).

Methods

Twenty subjects, 10 with SPT I-III, and 10 with SPT IV-VI were enrolled. Sites treated with three concentrations of a topical antioxidant complex (tocopherol, ascorbic acid, and diethylhexyl syringylidene malonate (and) caprylic/capric triglyeride) were compared with untreated control. The antioxidant complex was placed on participants' backs under occlusion for 1 hour followed by VL+UVA1 irradiation with 480 J/cm^2 for SPT 1-III, and 320 J/cm^2 for SPT IV-VI group. Clinical and colorimetric assessments were performed immediately, at 24 hours, and 7 days after irradiation.

Results

All 10 SPT I-III subjects had erythema response immediately after irradiation at all sites (treated and untreated). Colorimetry delta a* measurements demonstrate that the site treated with the highest concentration of the antioxidant complex had significantly lower erythema (p=0.007) compared to untreated control. All 10 SPT IV-VI subjects had an immediate pigment darkening response. Colorimetry delta ITA measurements demonstrate that the site that was treated with the highest concentration of the antioxidant complex was significantly lighter immediately after irradiation(p=0.005). At day 7, this trend continued although significance was not reached (p=0.07).

Conclusion

The VL+UVA1 doses used in this study, 480 J/cm^2 and 320 J/cm^2, correspond to approximately 2.5 and 1.5 hours of outdoor sun exposure, respectively. The results provide evidence that these doses induce biologic effects in subjects with all skin phototypes. The antioxidant complex reduced the intensity of the VL+UVA1 induced effects, supporting the hypothesis that by quenching reactive oxygen species, antioxidant products may mitigate these effects. Based on previous studies the protection offered, however, cannot be generalized to all antioxidants blends.

COI

This study was sponsored by Bayer.

Indermeet Kohli and Iltefat H Hamzavi are Investigators for Ferndale, Estee Lauder, Unigen, Johnson and Johnson, Allergan and Bayer, and are Consultants for Pfizer, Johnson and Johnson, and Bayer. Iltefat H Hamzavi is also an Investigator for Incyte. Henry W Lim is an Investigator for Estee lauder, Ferndale, Unigen, and Incyte and has served as a speaker in an educational session sponsored by Pierre Fabre. Alexis B Lyons and Raheel Zubair are investigators for Estee lauder, Unigen and Bayer. Amanda Nahhas and Taylor Braunberger are investigators for Ferndale, Estee lauder, Unigen and Bayer. Eduardo Ruvolo is a full-time employee of Bayer Healthcare LLC.

> OC026. Oral Communication
Symposium MED-15 Short Communications on Skin Photobiology (Yolanda Gilaberte)

A ROLE FOR VITAMIN D COMPOUNDS IN THE PREVENTION OF MELANOMA

Authors: Furkan A. Ince[1], Artur Shariev[1], Katie M. Dixon[1]
Presenting Author: Furkan Ince
1) Discipline of Anatomy and Histology, School of Medical Sciences and Bosch Institute, The University of Sydney, Sydney, NSW, Australia

Melanoma is the deadliest form of skin cancer and is responsible for 75% of all skin cancer deaths. In Australia, it is the third most common type of cancer in both men and women. Exposure to ultraviolet radiation (UV) from the sun can cause DNA damage in melanocytes and other skin cells, as well as immunosuppression, which together can lead to formation of melanoma and non-melanoma skin cancers. Vitamin D synthesis is initiated in the skin upon exposure to the UVB component of solar UV, and its conversion into active 1,25-dihydroxyvitamin D3 (1,25D) can take hours. Here, we confirm previous findings that 1,25D can reduce UV-induced DNA damage and cell death in human melanocytes and fibroblasts. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a known inhibitor of the oncogenic PI3K/AKT pathway. Previous studies demonstrated a reduction in PTEN levels in keratinocytes in response to UVB exposure. We now demonstrate a reduction in PTEN with solar simulated UV in melanocytes and dermal fibroblasts, and further show that 1,25D causes recovery of PTEN to pre-UV levels ($p < 0.05$, $p < 0.05$). We also confirmed this finding using *ex vivo* human skin samples, in which topical 1,25D also restored PTEN levels to pre-UV levels ($p < 0.05$). To further investigate the role of PTEN in the 1,25D-mediated photoprotection against UV-induced cell death of melanocytes and fibroblasts, we used siRNA for PTEN. We showed that the 1,25D-induced protection against UV-induced cell death was significantly reduced if PTEN had been silenced in these cells. Therefore, it appears that PTEN plays an important role in the photoprotective effects of 1,25D, and targeting of PTEN with 1,25D or other compounds may prove beneficial in the prevention of melanoma and non-melanoma skin cancers.

Acknowledgements
Human foreskin samples to culture melanocytes were kindly provided by Professor Andrew Holland.

Conflicts of interest
The authors have no conflicts of interest to declare.

References:

- Melanoma Institute Australia, 2017, 'Melanoma facts and statistics', viewed 3 August 2018, https://www.melanoma.org.au/understanding-melanoma/melanoma-facts-and-statistics/
- Dixon K M, Norman A W, Sequeira V B, Mohan R, Rybchyn M S, Reeve V E, Halliday G M & Mason R S 2011, '1α,25(OH)2-Vitamin D and a Nongenomic Vitamin D Analogue Inhibit Ultraviolet Radiation–Induced Skin Carcinogenesis', *Cancer Prev. Res.,* vol. 4, pp. 1485-1494.
- Sequeira V B, Rybchyn M S, Gordon-Thomson C, Tongkao-On W, Mizwicki M T, Norman A W, Reeve V E, Halliday G M & Mason R S 2013, 'Opening of Chloride Channels by 1a,25- Dihydroxyvitamin D3 Contributes to Photoprotection against UVR-Induced Thymine Dimers in Keratinocytes', *J. Invest. Dermatol.,* vol. 133, pp. 776-782.

> OC027. Oral Communication

Symposium MED-15 Short Communications on Skin Photobiology (Yolanda Gilaberte)

IN SILICO SIMULATION OF THE EFFECT OF SUNSCREEN ON DIRECT DNA DAMAGE IN DIFFERENT SKIN TYPES

Authors: Isla Barnard[1], Lewis McMillan[1], Ewan Eadie[2,3], Harry Moseley[2,3], Tom Brown[1], Kenny Wood[1]
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1) SUPA, School of Physics and Astronomy, University of St Andrews 2) Photobiology Unit, Ninewells Hospital and Medical School, NHS Tayside 3) Photobiology Unit, Ninewells Hospital and Medical School, University of Dundee

Introduction

Sunscreen, even at sub optimal application thickness, is known to prevent DNA damage within tissue (1). Different skin types, with different melanin content, exhibit different levels of DNA damage upon ultraviolet (UV) irradiation (2). Our previously published work has used *in silico* modelling to quantify DNA damage in the basal layer of skin caused by UV radiation (3). We modify this work to quantify the level of protection afforded by sunscreen against DNA damage in different skin types.

Methods

Monte Carlo radiative transfer (MCRT) methods use localised scattering and absorption probabilities to describe the path of photon packets through a medium. MCRT methods are ideally suited to modelling a complex structure such as the skin (4). A substance like sunscreen contains filters with well characterised optical properties, and as such, is suitable for MCRT modelling

A previously published MCRT skin model (3) was modified to include a layer of sunscreen. Irradiation of the sunscreen coated skin by solar UV radiation was simulated. This was repeated for different skin types and sunscreen formulations. The wavelength dependent fluence at depth achieved by the radiation is recovered, as is the amount of DNA damage occurring within the basal layer.

Results & Discussion

We find the multi layered MCRT model presented here reproduces previously published results (1,2) and we demonstrate the wavelength dependent protection sunscreen provides against DNA damage in the basal layer. Preliminary results also indicate the wavelength dependent nature of protection against DNA damage in the basal layer afforded to different skin types.

Conclusions

Multilayered MCRT may be a useful *in-silico* tool in modelling of sunscreen performance; allowing elucidation of wavelength dependent protection afforded to the basal layer of skin from the combined protection of sunscreen and the upper layers of skin.

Acknowledgements

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Photobiology Unit, Ninewells Hospital & Medical School, University of Dundee/ NHS Tayside, Dundee, UK

Conflicts of Interest

None

References

1. Young, A. R. et al (2018). Sub-optimal Application of a High SPF Sunscreen Prevents Epidermal DNA Damage in Vivo. *Acta dermato-venereologica*, *98*(9-10), 880-887.
2. Fajuyigbe, D. et al 2018). Melanin distribution in human epidermis affords localized protection against DNA photodamage and concurs with skin cancer incidence difference in extreme phototypes. *The FASEB Journal*, *32*(7), 3700-3706.
3. Barnard, I. R. M. et al (2018). Quantifying direct DNA damage in the basal layer of skin exposed to UV radiation from sunbeds. *Photochemistry and photobiology*, *94*(5), 1017-1025.
4. Wang, L. et al (1995). MCML—Monte Carlo modeling of light transport in multi-layered tissues. *Computer methods and programs in biomedicine*, *47*(2), 131-146.

> OC028. Oral Communication

Symposium MED-15 Short Communications on Skin Photobiology (Yolanda Gilaberte)

BROAD SPECTRUM PHOTOPROTECTIVE POTENTIAL OF THE MACROALGAE EXTRACTS FROM GRACILARIOPSIS TENUIFRON AND SARGASSUM SPP FROM COLOMBIAN CARIBBEAN

Authors: Stefanie Rincón Valencia[1], Yuliana Ospina Yepes[1], Juan Camilo Mejía[1], Miguel Ángel Puertas[1], Juan C. Scaiano[2]
Presenting Author: Stefanie Rincón Valencia
1) Universidad de Antioquia, Medellín, Colombia. 2) uOttawa, Ottawa, Canada

Introduction

Sun radiation contributes to the well-being of man by promoting the regeneration of cells and stimulating the production of vitamin D but it is widely reported that solar radiation, especially Ultraviolet Radiation (UVR), generates several adverse effects on the skin for example sunburn, free radical production, dehydration, photo-aging and worst of all photo-carcinogenesis. The use of sunscreens is the most common practice to protect against solar radiation. Most sunscreens available are mainly of synthetic origin, but certain disadvantages have been attributed, such as low photo-stability, active ingredients with narrow absorption in Ultraviolet range, systemic absorption and photo-contact dermatitis.

The compounds of natural origin have been an alternative because they exhibit broad ranges of absorption (UVA-UVB) and additional properties such as antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic. Reports confirm that algae have photo-protective properties as well as other benefits for skin care such as bleaching, antioxidant, anti-acne, antifungal, anti-aging and anti-allergic. [1-3]

Methods

The aim of this work was to evaluate the photoprotective potential of red algae *Gracilariopsis tenuifrons* and brown algae *Sargassum ssp* extracts. To achieve this, several extraction conditions were evaluated: solvent, assistance and time, the Relative Absorption Coefficient per gram of biological material was determined at 290, 310,340 and 380 nm. Later, a photo-stability study was made using a solar simulator Solsim and samples were taken at 0, 2, 4, 6 and 24 h. To complement results obtained the extract cytotoxicity in the cellular line of T3T fibroblasts was measured by MTT assay after 24 hours of incubation with different concentrations of the extract.

Results and Discussion

1 hour of ultrasound using water as solvent and 2 hours of ultrasound with a mixture Methanol: Water 50:50 were the methodologies that showed the greatest absorption in the UVA and UVB for brown algae and red algae respectively. The photostability showed that there is a decrease in the absorption of the extracts with the irradiation but after 24 hours there is significant absorption in the evaluated range. In both cases low cytotoxicity was found, for the red algae at 2 mg/mL the viability is 93.45% for the brown algae the highest cytotoxicity 80.38%. was presented at 1.5 mg/mL. According to these results both algae show potential to be used in photoprotection since their extracts exhibit absorption throughout the range of UVA and UVB, good photo-stability and low cytotoxicity.

Acknowledgments

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Bibliography

[1] J. C. Mejía-Giraldo., et al., *Derm. Cosm. Méd. Qui.*, 2014, 12(52), 272–281.
[2] K. P. Balakrishnan., et al., *Int. J. Re. Cosm. Scien.* 2011, 1(1), 1–12.
[3] M. Pandika., *ACS cent.*,2018, 4-788-790.

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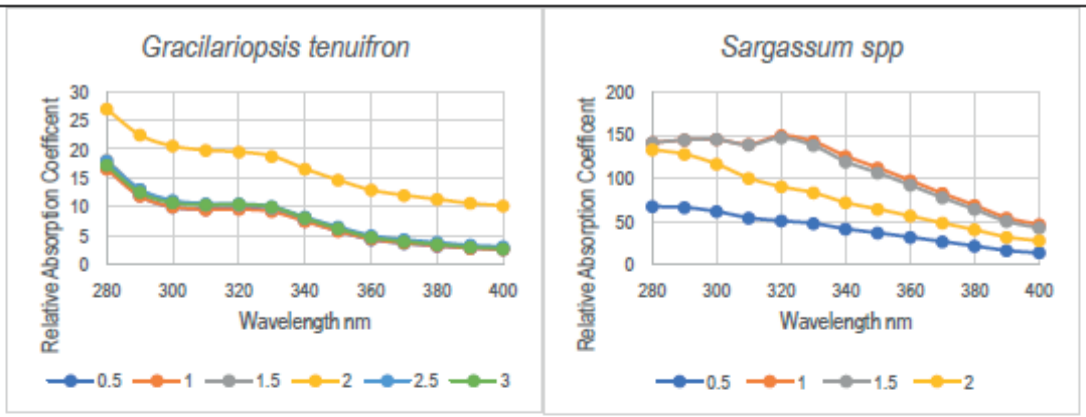

Figure 1: Relative Absorption Coefficient vs Wavelength at different times. The graph on the left corresponds to *Gracilariopsis Tenuifron* and the right to *Sargassum spp.*

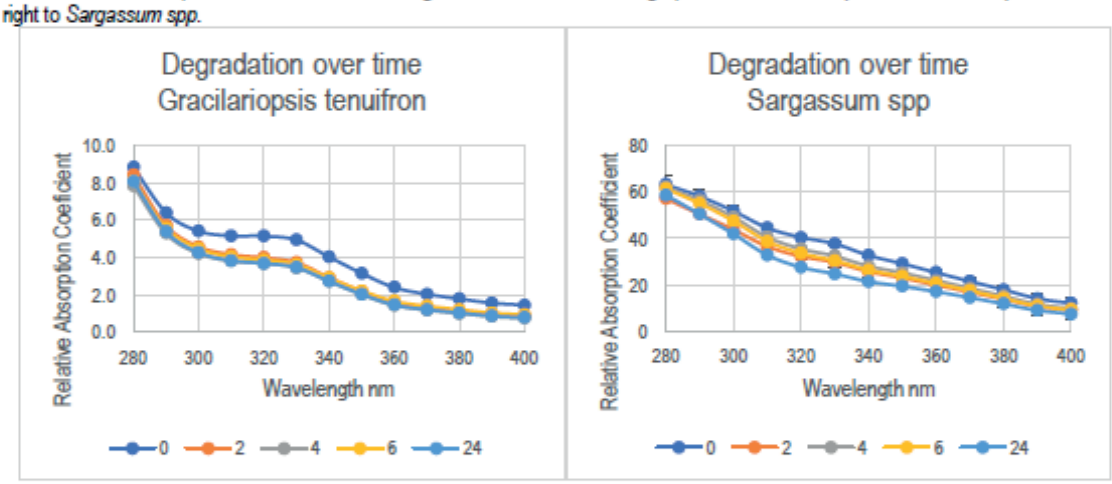

Figure 2: Relative Absorption Coefficient vs Wavelength at different irradiation times. The graph on the left corresponds to *Gracilariopsis Tenuifron* and the right to *Sargassum spp.*

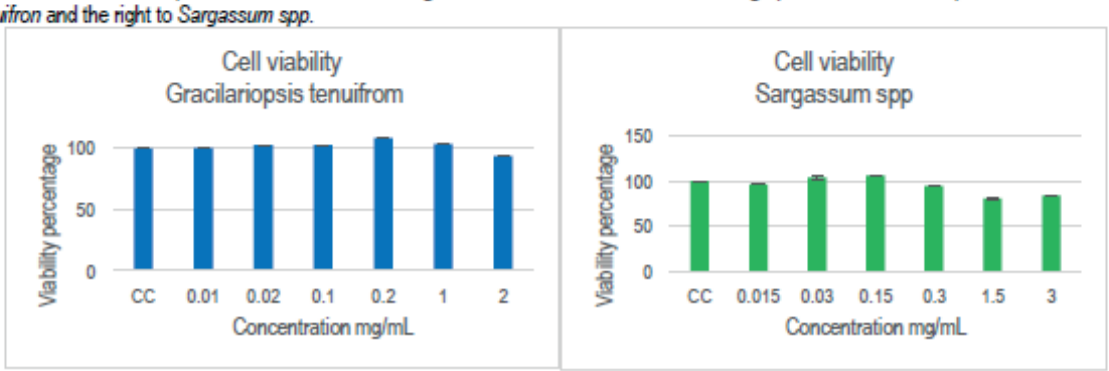

Figure 3: Cell viability at different extract concentrations. The graph on the left corresponds to *Gracilariopsis Tenuifron* and the right to *Sargassum spp.*

> OC029. Oral Communication

Symposium MED-15 Short Communications on Skin Photobiology (Yolanda Gilaberte)

THE CYTOPROTECTIVE POTENTIAL OF NOVEL MITOCHONDRIA-TARGETED IRON CHELATORS AGAINST UVA- AND HYDROGEN PEROXIDE-MEDIATED OXIDATIVE CELL DEATH IN FRIEDREICH'S ATAXIA FIBROBLASTS

Authors: Charareh Pourzand[1], Olivier Reelfs[1], Vincenzo Abbate[2], Agostino Cilibrizzi[2], Robert Hider[2]
Presenting Author:Charareh Pourzand
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We have previously demonstrated that the presence of labile iron (LI) in the mitochondria is a major contributor to the susceptibility of skin fibroblasts to ultraviolet A (UVA, 320–400 nm) component of sunlight. [1] This is because LI is recognised as a catalyst of oxidative damage in UVA-irradiated skin cells leading to necrotic cell death via ATP depletion. [2] We have further demonstrated that a mitochondria-targeted iron chelator developed by us, provides unprecedented protection against UVA-mediated oxidative cell death in skin fibroblasts. [1] Mitochondria iron overload represents a serious threat to cells' viability in the neurodegenerative disease Friedreich's ataxia (FRDA). Using cultured primary skin fibroblasts, we have recently shown that FRDA cells are in fact significantly more sensitive to UVA-induced death than their healthy counterparts. [3] Measurement of the mitochondrial LI using a sensitive iron sensor developed by us [4] reveals levels several-fold higher in FRDA cells than in healthy counterparts. Our results further demonstrated that the higher levels of mitochondrial LI in FRDA fibroblasts correlate with the higher generation of mitochondrial reactive oxygen species (ROS) as measured by the specific MitoSOX ROS indicator [3]. Here, we compared the cytoprotective potential of two newly developed bidentate and hexadentate mitochondria-targeted iron chelators (PD1, PD2) to that of clinically used iron chelators desferrioxamine (DFO) and deferiprone (DFP), in FRDA skin fibroblasts treated with oxidising agents UVA or H_2O_2. MTT and Annexin V/propidium iodide assays were used as cytotoxicity tests. Mitochondrial membrane damage and ATP depletion were monitored with TMRM labelling and ViaLight™ plus kit. The results show that FRDA cells are significantly more sensitive to both H_2O_2- and UVA-induced death than their healthy counterparts. Furthermore the novel mitochondria-targeted chelators abrogate the cell death mediated by both oxidising agents and significantly reduce oxidative damage to mitochondria. DFO was the least effective cytoprotective chelator. Our results highlight the potential of mitochondria-targeted iron chelators for the treatment of mitochondrial iron overload in FRDA.

References

1. Reelfs O, Abbate V, Hider RC and Pourzand C, J. Invest. Dermatol. 2016, 136, 1692-1700.
2. Aroun A, Zhong JL, Tyrrell RM, Pourzand C. Photochem. Photobiol. Sci. 2012, 11, 118-134.
3. Reelfs O, Abbate V, Cilibrizzi A, Pook MA, Hider RC, Pourzand C. Metallomics. 2019;11, 656-665
4. Abbate V, Reelfs O, Hider RC and Pourzand C, Biochem. J., 2015, 469, 357-366.

> IL375. Invited Lecture
MNK (Therakos) - Sponsored Satellite Symposium

PBL (PSORALENS + BLUE LIGHT): BLUE LIGHT ACTIVATES 8-MOP AND TMA TRIGGERING PROSTATE (DU145) AND VESICAL (T24) TUMOR CELL APOPTOSIS AND DEATH.

Authors: Giorgia Miolo[1], Luca Menilli[1], Alessia Tasso[1], Giulio Sturaro[1], Maria Teresa Conconi[1]
Presenting Author: Giorgia Miolo
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Introduction
Psoralens and angelicins (furocoumarins) are natural and synthetic compounds with high antiproliferative potency under UVA irradiation mainly used for the treatment of skin diseases (PUVA therapy) or immunological disorders in extracorporeal photopheresis (ECP). To improve their activity against psoriasis or vitiligo and avoid severe side effects mainly related to the formation of interstrand crosslinks (XLs) with DNA pyrimidine bases, a variety of derivatives, hopefully monofunctional, have been synthesized. Although angelicins, due to their angular geometry, do not generally form XLs, some of them, i.e. (TMA), can crosslink folded DNA upon UVA. Furthermore, furocoumarins produce ROS that impair cellular functions through lipid peroxidation, oxidation of guanine and strand breaks in nucleic acids, oxidation of proteins and inactivation of enzymes.

Methods
To photoactivate 8- MOP and 4,6,4\'-trimetylangelicin (TMA) towards human prostate (DU145 PCa) and bladder (T24) cancer cell lines, a new approach based on less toxic and more penetrating visible radiation (BL, 420 nm) is proposed.

Results and Discussion
TMA and 8-MOP showed high antiproliferative activity towards both cancer cell lines, through induction of apoptosis. Besides ROS generation (less efficient under BL than UVA), the proapoptotic effect seemed related to the activation of p38 and inhibition of p44/42 phosphorylation. Moreover, no phosphorylation of the histone H2AX, nuclear β -catenin and GSK3β occurred. Moreover, Cyclin D1, c-Myc and CD44v6 expression were reduced through inhibition of the Wnt pathway. Overall, DU145 cells appeared more sensitive to PBL than T24, showing a specificity of the test compounds towards different tumor cell lines. The strong photocytotoxicity of TMA and 8-MOP can be related to the kind and number of DNA lesions. Under BL, no mutagenic crosslinks, no photocleavage nor photooxidative lesions were detected on isolated DNA by TMA phototreatment, but only MAs can form. However, generation of XLs still remained for 8-MOP under BL but in a lower amount than under UVA.

Conclusions
Overall, our results indicate that 8-MOP, and particularly TMA, can be efficiently activated by BL and may be considered good candidates for targeted PBL of prostate and bladder cancers and possibly for other solid tumors.

> IL376. Invited Lecture

MNK (Therakos) - Sponsored Satellite Symposium

MECHANISMS OF ACTION – WHAT IS NEW IN ECP IMMUNOMODULATION

Authors: Peter Wolf[1]
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The exact mechanisms behind the therapeutic benefit ofextracorporeal photopheresis (ECP) have not been fully clarified, though it has been in clinical use for various diseases by now for more than 3 decades. The current view of the therapeutic mechanism of ECP favors cell killing by apoptosis, dendritic cell initiation, modification of cytokine and chemokine profile, and induction and/or stimulation of regulatory T-cells (Tregs). It is of particular interest that a malignant disease such as Sézary syndrome, a specific form of leukemic T-cell lymphoma, as well as inflammatory diseases such as atopic dermatitis, scleroderma, or graft-versus-host disease can all be effectively treated with ECP, as immunomodulatory therapy. A better understanding of the therapeutic mechanism of ECP shall in overall advance treatment strategies in the diseases treated with it.

SYMPOSIUM COMMUNICATIONS

PHOTODYNAMIC THERAPY

> IL087. Invited Lecture
Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

20 YEARS OF 5-ALA DERIVATIVES : LESSONS LEARNED AND NEW PERSPECTIVES

Authors: Norbert Lange[Unive]
Presenting Author: Norbert Lange
1) University of Geneva, School of Pharmaceutical Sciences

In the late 80's and earlier 90's of the previous century a new technology gained attention to the PDT community. 5-aminolevulinic acid (5-ALA) induced protoporphyrin IX (PpIX) excelled with outstanding selectivity for pathologic tissues. Since then, several clinical trials have been initiated that demonstrated this selectivity for a multitude of tumors including skin, bladder, and lung cancer after exogenous administration of 5-ALA. However, already early in the development of 5-ALA-mediated PDT and photodiagnosis, it became clear that 5-ALA itself had several drawbacks with respect to pharmacokinetics, biodistribution, stability, and PpIX generation.

To overcome these obstacles, research was first focusing on improved formulations of 5-ALA. However, it's only since the disclosure of more lipophilic 5-ALA derivatives that this branch of research obtained a new boost. Since then, two 5-ALA esters gained marketing authorization for the treatment of actinic keratosis and the detection of bladder cancer. Furthermore, 5-ALA hexylester is also used for skin rejuvenation. Further clinical trials for the treatment of other indications are currently ongoing. However, in most cases these compounds are restricted to topical use.

Here we will discuss briefly the history of 5-ALA esters and then discuss how the latter restriction can be circumvented.

> IL088. Invited Lecture
Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

DUAL FUNCTIONS OF ALA IN ACTIVATION OF PDT

Authors: Zvi Malik[Bar I]
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Topical ALA-PDT and fluorescence imaging of tumor have gained remarkable success after decades of research, and many mechanistic aspects were ascertained. Research indicates that no multidrug resistance develops as a consequence of PDT; this is of major significance in oncology. Onco- energy metabolism is anomalously manifested by aerobic glycolysis and the deregulated heme synthesis and catabolism. Consequently, the increased energy requirement for the rapid growth of tumors cannot be fulfilled by mitochondrial ATP supply due to the disturbed heme synthesis and accumulation of PpIX. Thus, ALA-PDT is based fundamentally on the deregulated heme synthesis pathway, which compels the accumulation of PpIX in neoplasms. Three basic aspects should be considered in this regard, the one, the means of ALA supply to tumor cells, second, the techniques to reduce ferrochelatase activity and the last, controlling the routes of cell death activated upon light irradiation.

We have shown that porphobilinogen deaminase (PBGD) activity is markedly enhanced by exogenous ALA treatment due to the dual function of ALA in the synthesis of PpIX. At the first phase of tumor treatment with ALA promote the condensation of 2 ALAs to form the crucial co-factor hydroxymethylbilane (DPM) by PBGD, which in turn activate dramatically its own enzymatic activity following covalent binding of DPM to the enzyme active site. During porphyrin synthesis, the enzyme forms stable covalent enzyme-substrate complexes with PBG, and the unique DPM cofactor binds the di- and tri-pyrrole intermediates at the active site until the formation of HMB is complete. Thus, the activated PBGD condensate 4 PBGs by a series of deaminations to form the linear tetrapyrrole, hydroxymethylbilane (HMB), and at the same time as HMB can cyclize non-enzymatically and enzymatically to form uroporphyrinogen.

Thus, ALA serves as a substrate for the DPM co-factor and a substrate for the tetrapyrrole rings and Pp IX. Finally, the rate-limiting mitochondrial enzyme ferrochelatase inserts a ferrous ion into the center of the PpIX molecule, an enzymatic step in neoplastic cells which is downregulated. The documented cell death directions stimulated by ALA-PDT are apoptosis and necrosis, much dependent on the total amount of accumulated PpIX in the tumor cell, the delivered light energy, and tumor origin. The critical function of ALA in the synthesis of PpIX is controlled by ALA delivery protocols and by various ALA- prodrugs, including molecules guiding deeper tissue penetration and other derivatives stimulating the porphyrin synthesis enzymatic pathway. Ferrochelates inhibition was shown to be an effective way of enhancing PpIX accumulation and controlling PDT efficacy, novel ALA prodrugs are designed in order to combine a dual function of ALA delivery in addition to iron chelation activity to reduce ferrochelatase activity.

> IL089. Invited Lecture

Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

STRATEGIES FOR ENHANCED INTRACELLULAR PORPHYRIN GENERATION AND PDT USING ALA DERIVATIVES

Authors: Sandy MacRobert[1]
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Photodynamic therapy (PDT) and photodiagnosis with 5-aminolevulinic acid (ALA) or its derivatives rely on metabolism of ALA to the photosensitiser, protoporphyrin IX, (PpIX). However, the efficacy of ALA to PpIX conversion is limited by several factors including relatively poor ALA cellular uptake and limited intracellular accumulation of PpIX owing to its conversion to haem which is catalyzed by ferrochelatase. To improve cellular uptake ALA esters have been investigated, including dendrimeric ALA ester derivatives.[1] In the case of dendrimers, the rate of ALA release is generally slower and sensitive to the length of the ester linkage. Other approaches include dipeptide ALA conjugates. [2] To address the effect of ferrochelatase, hydroxypyridinones (HPO) have been investigated as they are efficient biocompatible inhibitors of ferrochelatase. Co-administration with ALA can amplify PpIX generation both in vitro and in vivo. In a modified approach, single or dendrimeric conjugates of ALA and an HPO chelator, covalently bound via a biodegradable linkage, can be used to enhance PpIX levels. The efficacy is found to depend strongly on the linkage design employed to conjugate ALA with the HPO. The higher PpIX levels observed using the conjugates correlate well with the increased phototoxicity observed following exposure of cells to light.

References

1. Fluorescence diagnosis of bladder cancer: a novel in vivo approach using 5-aminolevulnic acid (ALA) dendrimers. François A, Battah S, MacRobert AJ, Bezdetnaya L, Guillemin F, D\'Hallewin MA. BJU Int. 2012 110:E1155-62
2. Chemical approaches for the enhancement of 5-aminolevulinic acid-based photodynamic therapy and photodiagnosis. Tewari KM, Eggleston IM. Photochem Photobiol Sci. 2018 17(11):1553-1572. doi: 10.1039/c8pp00362a.

> IL090. Invited Lecture
Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

RESPONSE TO ALA-PDT IN 3D OVARIAN CANCER AND IN VIVO MODELS OF PERITONEAL DISSEMINATION

Authors: Mariela Céspedes[1], Gabriel Orlando[1], Gustavo Calvo[1], Agustina Taruselli[2], Gabriel Gola[3], Javier Ramírez[3], Tayyaba Hasan[4], Daniel Sáenz[1], Gabriela Di Venosa[1], Alejandro Urtreger[2], Adriana Casas[1]
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ALA-PDT is an effective therapy mainly for dermatologic cancer, although its uses have been extended in the last years. PDT with benzoporphyrin derivatives has been employed in the treatment of ovarian cancer [1]. In addition, Photodiagnosis with ALA for intraoperative detection of peritoneal metastases of ovarian cancer has been proposed [2].

When ALA is administered systemically, tumor selective production of Protoporphyrin IX is observed. One approach to broaden clinical ALA-PDT uses is the design of derivatives of ALA with the aim of improving bioavailability and selectivity.

Three-dimensional culture models (spheroids) have proven to be a realistic scenario to test response to ALA-PDT, where physical variables reduce tumor response to treatment. On the other hand, 3D tumorspheres are enriched in stem-like cells, which are related to metastasis and resistance. Since ovarian cancer frequently presents peritoneal dissemination, the use of 3D models mimicking peritoneal metastasis, constitutes an interesting approach.

The aim of this study was to gain insight in the response of ovarian cancer 3D models to PDT employing ALA or new derivatives, and to analyze its selectivity for tumor tissue in vivo.

Materials and methods: SKOV-3 and IGROV-1 human ovarian cancer cell lines were employed. SKOV-3 spheroids were used as metastatic nodules model and IGROV-1 tumorspheres, as tumor stem cells model. Athymic mice N:NIH (S)-Fox1 nu were injected i.p. with SKOV-3 cells to induce peritoneal dissemination. White lamps and a 630 nm laser (Lumiia, Argentina) were employed as light sources. ALA and ALA derivatives synthesised by multicomponent reactions [3] were employed.

IGROV-1 tumorspheres overexpressed NANOG, OCT4 and SOX2 pluripotent genes [4] and exhibited 2-fold increase of resistance to ALA-PDT as compared to 2D cultures, thus suggesting a role of ovarian stem cells in the resistance to PDT.

The role of the 3D structure on the resistance was studied in spheroid SKOV-3 cultures. They proved to be resistant to ALA-PDT employing low power non-coherent light sources. However, they were responsive to the treatment employing a coherent red light source. In addition to increasing light dose, the other approach to revert resistance to PDT was the use of more lypophilic ALA compounds, of which the so called 89-ALA was the one which better penetrate the spheroid structure.

When 89-ALA was injected into mice with i.p. dissemination of SKOV-3 cells, fluorescence was much more confined to the tumor spots in comparison to ALA.

The use of ALA derivatives improves ALA-PDT performance in ovarian cancer. Our results reinforce the importance of the studies of pro-photosensitizer penetration and PDT response in 3D models as a previous step to animal studies.

References

[1] del Carmen et al. *J Natl Cancer Inst.* 97:1516-24(2005).
[2] Hillemanns et al. Lasers Surg Med. 49:169-176(2017).
[3] Gola et al. *RSC Adv.* 6, 89492-98(2016).
[4] Li et al. *Chin J Cancer*. 32:483-7(2013).

> IL091. Invited Lecture

Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

OPTIMISATION STRATEGIES FOR ALA-PDT OF NON-MELANOMA SKIN CANCER: RESULTS OF A TRANSLATIONAL RESEARCH APPROACH

Authors: Ben Novak[1,2], Luisa Heesen[2], Nicole Schary[2], Lutz Schmitz[3], Ann-Kathrin Hoeh[4], Thomas Dirschka[4], Mirella Gwarek[1], Montserrat Foguet[1], Beate Schmitz[1], Hermann Luebbert[1,2]
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Introduction

5-aminolevulinic acid based photodynamic therapy (ALA-PDT) has long been proven clinically useful for a variety of skin diseases, most notably epidermal neoplasia. However, clinical application indicates that further improvement is desirable to best meet patients' needs. We developed a preclinical research programme to better understand critical success factors such as prodrug stability, skin penetration, treatment emerged pain, the influence of illumination parameters, and treatment resistance. Here we describe how a translational research programme can inform clinical development and how early discoveries can be mapped to therapeutic reality.

Methods

- We characterised the impact of a nanoemulsion-based drug delivery system (BF-200) to improve ALA stability and penetration, as investigated in cell cultures, nude mice and minipig *in vivo*, and porcine and human skin *in vitro*.
- Primary sensory neuron cell cultures were treated with ALA *in vitro* to understand pain mechanisms in the skin.
- Squamous cell carcinoma (SCC) cell lines were analysed for biochemical aspects of ALA uptake and PpIX formation as well as resistance mechanisms and the influence of different illumination parameters on photodynamic efficacy.
- Another line of investigation covered the interaction of ALA-PDT with putative cancer stem cell subpopulations from SCC cell lines.

Results

- Combining ALA with BF-200 highly increased stability and penetration into cell cultures porcine and human skin and was superior to both a methyl-ALA cream and ALA in standard galenic vehicle.
- We could identify both direct and indirect mechanisms of cutaneous sensory neuron activation by PDT, giving rise to potential analgesic targets.
- We discovered differential gene expression of ALA uptake transporters and heme synthesis enzymes in two SCC lines that show different PpIX formation kinetics and phototoxic response profiles, which uncovered potential resistance mechanisms. In the same cell lines, we found that total light dose is the key factor for efficacy as opposed to fluence rate.
- A stemness associated gene expression panel was implemented to investigate the susceptibility of cancer stem cells to PDT *in vitro*.

Conclusion

ALA stability and penetration were significantly enhanced by the nanoemulsion BF-200. The results from the different *in vitro* approaches can be translated into future development programmes to further improve efficacy and tolerability of ALA-PDT.

Conflict of interest

HL, BN, BS, MG & MF are employees of the Biofrontera group which developed an ALA containing drug and a PDT light source. LS, AKH, LH, NS & TD declare no conflict of interest.

> OC030. Oral Communication
Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

TARGETING THE ULTRAVIOLET A-INDUCED LABILE IRON RELEASE TO IMPROVE THE EFFECTIVENESS OF TOPICAL AMINOLEVULINATE-BASED PHOTODYNAMIC THERAPY OF SKIN CELLS

Authors: Dana Beiki[Unive], Olivier Reelfs[Unive], Ian Eggleston[Unive], Charareh Pourzand[Unive]
Presenting Author: Dana Beiki
1) University of Bath

Topical aminolevulinate-based photodynamic therapy (ALA-PDT) is recognised as an effective treatment for actinic keratoses. Application of ALA causes the accumulation of photosensitising concentrations of protoporphyrin IX (PpIX) which following irradiation with blue or red light catalyses the generation of reactive oxygen species, resulting in cell death. A major drawback of topical ALA-PDT is the pain experienced by patients that may cause non-compliance and termination of the course of the treatment. To improve the efficiency of ALA-PDT of skin cells, we have applied two approaches. (i) We changed the conventional light source to UVA (320-400 nm) which is absorbed more efficiently by PpIX than red light and is 40-fold more potent in killing skin cells [1,2]. (ii) We attempted to exploit the damaging effects of rapid release of labile iron by applying short pulses of low UVA instead of a continuous source of light following ALA treatment. This is because the labile iron released in ALA-treated cells after the first irradiation acts as a catalyst to exacerbate the oxidative damage upon subsequent exposures [3-5]. HaCaT keratinocytes were treated with two therapeutic doses of 0.5 and 1 mM ALA for 2 h and then irradiated with a range of UVA doses of 0.1-0.5 J/cm^2 with 1 or 2 h dark intervals. The UVA doses are equivalent to 0.5-1.5 min sunlight. In clinical settings these are short pulses of ca 5-25 s. Cell death was examined 24 h after UVA by MTT, annexin V-propidium iodide and colony forming assays. The level of labile iron was measured with the fluorescent calcein assay. The results showed that both ALA concentrations significantly increased the level of PpIX and sensitized keratinocytes to very low non-cytotoxic UVA doses. The calcein assay revealed a higher level of labile iron release after UVA-irradiation of cells treated with 1 mM ALA. The latter correlated with higher accumulation of PpIX and increased sensitivity to UVA-irradiation of cells treated with 1 mM ALA. Among all the conditions tested, applying short pulses of UVA (i.e. 1 and 2.5 kJ/m2) to 1 mM ALA-treated keratinocytes with a 1 h dark interval was found to be the most effective way to promote cell death. The latter may be used to improve the current modality for topical ALA-PDT, through a reduction of the irradiation time and thus the length of pain likely to be endured during the treatment. References: [1] Buchczyk et al, Carcinogenesis 2001, 22:879-883. [2] Pourzand et al, J. Invest. Dermatol. 1999, 112:419-425. [3] Pourzand et al, Proc. Natl. Acad. Sci. USA 1999, 96:6751-6756; [4] Zhong et al, J. Invest. Dermatol, 2004, 123:771-780; [5] Reelfs et al, J. Invest. Dematol 2016, 136:1692-1700.

> OC031. Oral Communication
Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

SERVICE IMPROVEMENT AND RESEARCH TO INCREASE CONFIDENCE IN DAYLIGHT PHOTODYNAMIC THERAPY DELIVERED IN THE UK

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Introduction
Daylight Photodynamic Therapy (dPDT) is a patient preferred, effective and well-tolerated treatment for Actinic Keratoses (AK). The Photobiology Unit at Ninewells Hospital in Dundee, Scotland, was an early adopter of this therapy in the UK. To increase confidence in dPDT and improve clinical delivery a portfolio of service improvement and research activities, mainly focussed around patient priorities and light delivery, has been undertaken.

Methods
Patient engagement was the initial step in service improvement. In order to better understand the views of our stakeholders, 56 patients who had previously undergone dPDT were mailed a 19 question survey. Of the 35 respondents, nine patients with differing views were subsequently invited to an engagement event. In parallel with these service improvement activities, a research strategy was developed and implemented in collaboration between the Photobiology Unit and Public Health England. Large quantities of historical ultraviolet and visible light data were interrogated to investigate the viability of dPDT in the UK, supported by a three-year retrospective analysis of clinical data.

Results and discussion
82% of questionnaire respondents said they were happy or very happy with dPDT [1]. The most important objectives for patients were: minimum discomfort, high levels of disease improvement, cosmetic outcome and convenience of therapy. 59% thought dPDT was better than most or all other therapies for AK and most (80%) were able to tolerate it at least as well as alternatives used [1]. Historical measurement data demonstrated that a minimum light-dose threshold for effective dPDT can be achieved from spring to autumn, across the UK, extending into winter if conservatory treatment is considered [2]. In addition, this minimum threshold light-dose was demonstrated to be achieved at times of the day (late afternoon in summer) and months of the year (October to March) when erythemal ultraviolet (UV) exposure is minimised (< 4 standard erythemal doses). The effective dPDT light dose received was also found to be highly dependent on the sunscreen used during treatment, with up to a 65% dose reduction when frequently used organic filter sunscreens were applied [3]. Our scientific results are supported by our retrospective clinical data, which show 73% clearance or good response with very low pain scores (median 1 on a visual analogue scale of 0 to 10) with dPDT [4].

Conclusion
There is enough light available throughout most of the year in the UK to deliver effective dPDT. We would advocate increased uptake of this patient preferred AK treatment in the UK.

Conflicts of Interest
L.M. is funded by Innovate UK, P.O'M. is funded by the Medical Laser Research Fund (SC037390). P.O'M, E.E. and SI have received conference expenses from Galderma.

References

1. McLellan, L.J. et al. Br. J. Dermatol. 2019 (accepted online).
2. O'Mahoney, P et al. Br. J. Dermatol. 2017; 176(6):1607-1616. https://doi.org/10.1111/bjd.15146
3. O'Mahoney, P. et al. Br. J. Dermatol. 2019 (accepted online). doi.org/10.1111/bjd.17895
4. Cordey, H. et al. Scot. Med. J. 2017; 62(2):48-53. https://doi.org/10.1177/0036933017695156

> OC032. Oral Communication

Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

SmartPDT: SMARTPHONE-ENABLED DAYLIGHT PDT BASED ON SOLAR RADIATION DOSIMETRY USING EARTH OBSERVATION SATELLITES

Authors: Luke McLellan[1], Marco Morelli[2], Marina Khazova[3], Emilio Simeone[2], Sally Ibbotson[1,4], Ewan Eadie[4]
Presenting Author: Luke McLellan
1) The University of Dundee 2) siHealth Ltd 3) Public Health England 4) NHS Tayside, Ninewells Hospital and Medical School

Introduction

Daylight photodynamic therapy (dPDT) is an effective, patient-preferred field-directed treatment for actinic keratosis (AK).[1,2] A topical pro-drug is applied to the affected skin, is taken up and metabolised to the photosensitiser protoporphyrin IX (PpIX), which preferentially accumulates in dysplastic and neoplastic cells. Under visible solar radiation, PpIX is photo activated in the presence of oxygen to initiate oxidative stress and a cascade of events resulting in AK clearance. Currently, there is no standardised or reliable method for light dosimetry during dPDT. It is therefore not possible to determine and influence the quantity of light a patient receives, nor is it possible to ensure that the required minimal PpIX dose for effective treatment has been achieved.

Methods

SmartPDT is an innovative application developed by siHealth Ltd in the frame of an Innovate UK-funded project performed in collaboration with the University of Dundee. It is a mobile app aimed to assist dPDT by providing real-time monitoring of PpIX-weighted dose from Earth Observation satellite imagery. It also forecasts the PpIX-weighted dose by using archive satellite data and Numerical Weather Predictions to support treatment planning. We retrospectively compared satellite-derived data with ground-based spectral measurement provided by Public Health England at two locations (Dundee and Chilton, UK) between the months of May and October 2017. 48 hour and 24 hour forecasted PpIX-weighted dose derived from the application were also prospectively compared to the dose measured by ground stations.

Results and discussion

A direct comparison between ground and satellite PpIX-weighted dose data showed excellent correlation (Chilton R^2 = 0.92, Dundee R^2 = 0.90). PpIX-weighted dose for three distinct time periods (30 minutes, 1 hour and 2 hours) demonstrated average percentage differences between satellite and ground measurements of -7.5%, -7.5% and -6.8% for Chilton and -13.6%, -13.5% and -12.3% for Dundee. A similar comparison has been also performed on preliminary forecasted data, showing a good correlation for 24 hours forecasted dose (Chilton R^2 = 0.86, Dundee R^2 = 0.84) and for 48 hours forecasted dose (Chilton R^2 = 0.75, Dundee R^2 = 0.76).

Conclusion

The SmartPDT application provides excellent agreement with ground-based measurements of PpIX-weighted dose and has the potential to deliver real-time dosimetry for patients undergoing dPDT, as well as providing essential support for both patients and clinicians with respect to dPDT treatment planning.

Conflicts of Interest

L.M. is funded by Innovate UK, M.M. is the CTO and E.S. is the CEO of siHealth Ltd.

References

1. Wiegell SR et al., Br. J. Dermatol. 2008; 158: 740-6
2. Lacour JP et al., J. Eur. Acad. Dermato Venereol. 2015; 29: 2342-8

> OC033. Oral Communication

Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

INVESTIGATION OF 5-ALA/5-FU COMBINATION THERAPY USING NIR-EMITTING Ag2S QUANTUM DOTS

Authors: Gözde Demirci[1], Mahshid Hashemkhani[1], Layla Mohammad Hadi[3], Ali Bayır[2], Abdullah Muti[4], Alphan Sennaroğlu[1,2,4], Marilena Loizidou[3], Alexander J. MacRobert[3], Havva Yagcı Acar[1,2,5]
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1) Graduate School of Material Science and Engineering, Koc University, Turkey 2) Department of Chemistry, Koç University, Turkey 3) Division of Surgery and Interventional Sciences, University College of London, England 4) Department of Physics and Electrical-Electronics Engineering, Koc University, Turkey 5) Surface Sciences and Technology Center (KUYTAM), Koc University, Turkey

Introduction

5-Fluorouracil (5-FU) is the most common chemotherapeutic agent used in colorectal cancer. Yet, its targeted delivery to reduce the side effects and enhance the therapeutic outcome is still needed. Besides, photo-dynamic therapy (PDT) is a therapeutic technique, wherein a photosensitizer is excited with an external light and produce a high local concentration of reactive oxygen species (ROS) to drive cells to apoptotic death. 5-Aminoleuvionic acid (ALA) is a popular pro-drug for PDT. It is converted to PpIX, the photosensitizer, and produces ROS when excited at 400 or 630 nm. For the delivery of the therapeutic agents (drugs or photosensitizers) usually nanoparticles are effective delivery vehicles. Ag_2S quantum dots (QD) are popular nanoparticles suitable for optical imaging in the medical imaging window. Ag_2S QDs coated by 2-mercaptopropionic acid (2MPA) have been used for targeted drug delivery before [1, 2] and proven as a cyto/hemocompatible quantum dots. Here, we will discuss the Ag_2S QDs conjugated with 5FU and ALA for enhanced therapeutic outcome in 2D and 3D *in vitro* experiments via combination therapy.

Methods

2-MPA coated Ag_2S QDs have been synthesized as our previous report [2]. Hydroxylated 5-FU[3] was conjugated to QD via an ester linkage. ALA was loaded electrostatically in HEPES solution. *In vitro* studies performed with colorectal cancer (HT29) cell lines. *In vitro* PpIX was determined after incubation of ALA or ALA loaded QDs. Dark cytotoxicity of each component and cell death after laser irradiation at 630 nm was determined.

Results & discussion

2-MPA coated Ag_2S QDs showed no significant toxicity to HT29 cell line however QD-5FU is more toxic than free 5FU (Fig.1). When ALA was loaded, the cytotoxicity of Ag_2S-5FU/ALA was similar with free 5FU (Fig.1). We have also studied the ALA to PpIX conversion *in vitro*. Co-loading of 5FU with ALA reduced the PpIX formation, especially at high concentrations, suggesting a possible interaction between the two (Fig.2). This was also confirmed via co-incubation of 5FU and ALA to cells without QDs and by using a synthesized 5FU-ALA conjugate. We will discuss the therapeutic outcome of ALA and 5FU loaded QDs after PDT experiments, as well.

Conclusions

We have developed 5FU conjugated and ALA loaded Ag_2S QDs for combination therapy. 5FU conjugated QDs are promising in reducing the effective dose of 5FU for therapy. Yet, 5FU interferes with the ALA to PpIX conversion. At low concentrations effective combination therapy can be achieved with co-loaded Ag_2S QDs.

Financial aid

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Continued

References

[1] Duman FD, et. al. Nanoscale **2015**; 7: 11352-62.
[2] Hocaoglu I, et. al. J. Mater. Chem. **2012**; 22: 14674-81
[3] Ouyang L et al. Bioorg. Med Chem. **2011**;19(12): 3750-56.

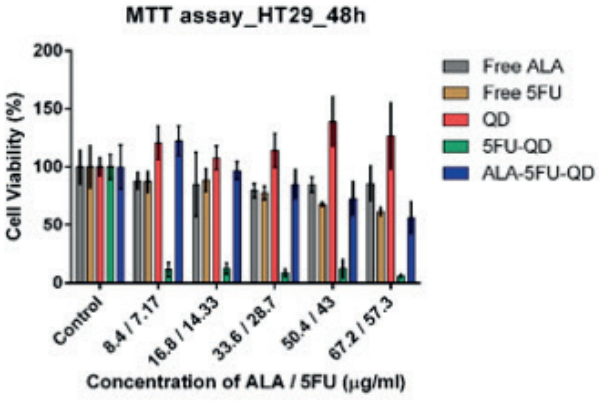

Figure 1: MTT assay results

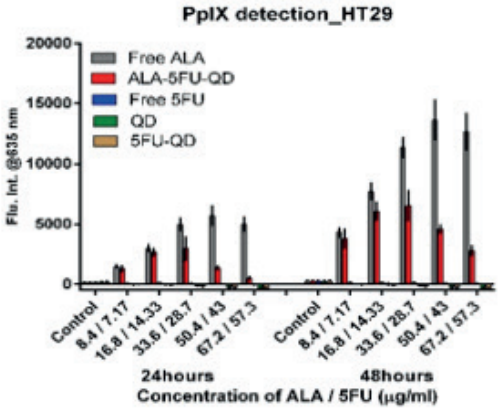

Figure 2: PpIX detection

> P024. Poster

Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

ENHANCEMENT OF PHOTODYNAMIC THERAPY CONDUCTED IN CULTURED HUMAN CELLS WITH A NOVEL COMBINATIONAL IRON CHELATING AGENT AND PROTOPORPHYRIN IX PRODRUG

Authors: Alison Curnow[1], Charlotte Reburn[1]
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Introduction

Superficial cases of non-melanoma skin cancer can be treated effectively and with excellent cosmesis with protoporphyrin IX (PpIX) photodynamic therapy (PDT)[1]. A prodrug aminolaevulinic acid (ALA) or its methyl ester (MAL) is applied to the skin and left occluded for 3h to allow the photosensitising agent PpIX to accumulate via innate haem biosynthesis prior to irradiation with red light. The efficacy of PpIX-PDT is decreased by Fe^{2+}, which binds to PpIX to form haem, and so this conversion can be reduced and PDT efficacy enhanced by adding an iron chelating agent[2].

Methods

A novel drug, AP2-18, consisting of the iron chelator CP94, ester-bound to ALA has now been synthesised and is being investigated experimentally[3,4]. A range of clinically relevant primary human cell types were cultured and utilised to try to mimic the clinical PDT process. Cells were incubated with ALA and MAL +/- CP94 or AP2-18 and PpIX fluorescence recorded up to 6h. Cell death was assessed via neutral red uptake and lactate dehydrogenase release 16h after PDT was conducted with 37 J/cm^2 635 nm light.

Results and Discussion

Both separate administration of the iron chelating agent CP94 and the new combinational iron chelator, AP2-18 were found to significantly increase PpIX accumulation in epidermal squamous carcinoma cells beyond that achieved by the standard prodrugs alone. This increased fluorescence translated to increased cell kill on irradiation without significant dark cytotoxicity being observed. Iron chelation clearly improved PpIX-PDT effectiveness by permitting more PpIX to accumulate during the drug light interval, resulting in increased production of reactive oxygen species on irradiation. AP2-18 is advantageous over the separate administration of CP94 as its design ensures delivery of the iron chelator and PpIX prodrug to cells simultaneously, for maximum effectiveness.

Conclusions

The combinational iron chelator and PpIX prodrug, AP2-18 has the potential to improve the effectiveness of dermatological PDT and should be investigated *in vivo*.

Acknowledgements

Medical Research Council, UK and Killing Cancer, UK for financial support. Dr Alexis Perry and Dr Mark Wood (University of Exeter) for synthesising AP2-18. Dr Anette Magnussen and Lizette Anayo for preliminary experimentation.

Conflicts of Interest

A. Curnow, A. Perry and M. Wood have patent PCT/GB2013/052297 issued.

References

[1]Morton *et al*. European Dermatology Forum Guidelines on topical PDT. Eur J Dermatol. 2015;25:296-311. [2]Curnow & Pye. The importance of iron chelation and iron availability during PpIX-induced PDT. Photonics Lasers Med. 2014;4:39-58. [3]Anayo *et al*. An experimental investigation of a novel iron chelating protoporphyrin IX prodrug for the enhancement of PDT. Lasers Surg Med. 2008;50:552-65. [4]Curnow *et al*. Improving in vitro PDT through the development of a novel iron chelating aminolaevulinic acid prodrug. Photodiag Photodyn Ther. 2019;25:157-65.

> P025. Poster

Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

EXPRESSION OF STEMNESS-ASSOCIATED GENES IN SQUAMOUS CELL CARCINOMA CELLS AFTER PHOTODYNAMIC THERAPY

Authors: Luisa Heesen[1, 2], Felix Krause[1], Ben Novak[1, 3], Hermann Lübbert[1, 4]
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Cancer stem cells (CSC) are considered to be responsible for maintenance, metastasis and recurrence of tumours after treatment as they are often resistant to different treatment options. Photodynamic therapy (PDT) is a common method of treatment for actinic keratosis, the precursor of squamous cell carcinoma (SCC). To this day, publications dealing with the efficacy of devitalizing CSCs by using PDT are very contradictory. Further investigations on the impact of PDT on CSCs are crucial for a better understanding of treatment efficacy and disease recurrence. One possible approach is to characterise PDTsurviving cells with regard to their CSC biomarker expression profile.

In this study, we compare the expression of CSC biomarkers in two squamous cell carcinoma cell lines, A431 and SCC13, prior to and after PDT on the mRNA level. Previous research of our group could show that the SCC-13 cell line is in general more resistant against PDT than the A431 cell line. But whether this is causally related to the size of the CSC subpopulation in these cell lines has not been established yet.

A variety of genes are known to be associated with stemness of cancer cells. Quantitative real-time polymerase chain reaction (qPCR) is a well-established method to test various cell populations for the expression of many different CSC-markers at the same time. An extensive gene panel could be established to assay PDT survivor cells for stemness, in comparison to untreated controls. Among others, the panel contains target genes like *ALDH1A1*, *integrin α6*, *CD44* and further genes associated with epithelial-mesenchymal transition, angiogenesis, differentiation and embryonic development.

> P026. Poster

Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

BYSTANDER EFFECTS OF NITRIC OXIDE IN MODEL SYSTEMS OF ANTI-TUMOR PHOTODYNAMIC THERAPY

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Tumor cells exposed to stress-inducing radiotherapy or chemotherapy can sent signals to non- or minimally-exposed counterparts (bystander cells). While bystander effects of ionizing radiation are well established, much less is known about them in the case of non-ionizing photodynamic therapy (PDT). In previous work, we showed that various breast, prostate, and brain cancer lines strongly upregulated inducible nitric oxide synthase (iNOS) and nitric oxide (NO) after a moderate 5-aminolevulinic acid (ALA)-based PDT-like challenge. The NO played a key role in cell resistance to photokilling as well as greater growth and migration/invasion aggressiveness of surviving cells. Based on this work, we hypothesized that diffusible NO produced by PDT-targeted cells in a tumor might elicit pro-growth/migration responses in non-targeted bystander cells. We recently tested this hypothesis using a novel approach in which ALA-PDT-targeted human cancer cells (prostate PC3, breast MDA-MB-231, or melanoma BLM) on large culture dishes were segregated from non-targeted bystanders via impermeable silicone-rimmed rings. At some interval (e.g. 20 min.) after the dishes were irradiated (fluence ~1 J/cm^2), rings were removed, and both cell populations were analyzed for various post-hv responses. Using immunoblotting, we observed a post-hv upregulation of targeted cell iNOS in this order: PC3 > MDA-MB-231 >> BLM, and this was reiterated in bystander cells. Bystander cells also grew and migrated faster than controls according to the same general order. Each bystander response was strongly suppressed by an iNOS inhibitor or NO scavenger, indicating that targeted cell iNOS/NO was responsible - the greater its induction by photostress, the greater the bystander response. These findings suggest that a feed-forward field effect of NO was in operation. If occurring in an actual tumor after PDT, this effect could compromise treatment efficacy or possibly even stimulate disease progression if cell targeting and eradication is not great enough. Supported by MCW Cancer Center grants FP12605 and FP14869 (to A.W.G) and NCN grant 2017/26/M/NZ3/01232 (to W.K.).

> P027. Poster

Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

EXPRESSION OF HEME SYNTHESIS ENZYMES, ALA UPTAKE AND PpIX EFFLUX TRANSPORTERS IN TWO SKIN CANCER CELL LINES WITH DIFFERENT DEGREES OF PDT RESISTANCE

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Photodynamic therapy (PDT) is a minimally invasive treatment widely used in actinic keratosis and non-melanoma skin cancer (NMSC). The three major components of PDT are a photosensitizer, light and oxygen. 5-aminolevulinic acid (5-ALA) is a metabolic precursor of Protoporphyrin IX (PpIX), a common photosensitizer in PDT. Protoporphyrin IX (PpIX) occurs as a natural intermediate in heme synthesis, which is regulated by the enzymes of the heme biosynthetic pathway. An external addition of 5-ALA leads to a selective accumulation of PpIX in tumor cells, since these cells upregulate the enzyme Porphobilinogen deaminase (PBGD) and downregulate the enzyme ferrochelatase (FECH). Despite the high efficiency of PDT, resistance of tumors and recurrence have been described and the mechanisms underlying this are not fully understood.

A431 and SCC-13 are cutaneous squamous-cell carcimona (cSCC) cell lines in which previous work at our department could show that SCC-13 are more resistant to PDT than A431. However, this PDT resistance in SCC-13 decreased with increaseing passage number. Quantification of PpIX in the cell lines after exposal to equal 5-ALA concentrations and incubation times, implied that different PpIX formation capacity may be a key factor for PDT resistance.

The main goal of the experiments presented here was to investigate which enzymes and transporter may lead to a decreased formation of PpIX and thus to PDT resistance.

Therefore, gene expression of 5-ALA uptake transporters, heme synthesis enzymes and PpIX efflux transporters was investigated by quantitative real-time PCR in A431 and SCC-13.

It could be established that SCC-13 cells express PEPT-2 as their sole 5-ALA uptake transporter in contrast to A431 cells, which express PEPT-2 together with GAT-3.

Additionally, the expression of the enzymes UROD and CPOX and the PpIX efflux transporter ABCG2 in SCC-13 cells was differential in contrast to A431.

Further, pharmacological inhibition experiments of ALA uptake transporters could show that these two transporters are the main ALA uptake routes in the cell lines. PpIX amount could also be increased via inhibition experiments of PpIX efflux transporters or modulation of gene expression of heme biosynthetic pathway.

The findings of this work could be relevant for the therapeutic use of PDT as they might provide insight into mechanisms that govern disease recurrence and resistance.

> P028. Poster

Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

ENLARGING THE SCOPE OF 5-AMINOLEVULINIC ACID PHOTODIAGNOSIS TOWARDS BREAST CANCER

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Introduction

In 2018, breast cancer (BC) was the most commonly diagnosed cancer in women with more than 2 million new cases and the first cause of female cancer death (>625,000). Mammography being the only effective screening method, researches started to extend photodiagnosis (PD) to breast cancer.

Protoporphyrin IX (PpIX) is a natural molecule whose production has been widely browsed for photodynamic therapy (PDT) and PD applications due to its photosensitizing potential. Synthesis of this ultimate heme precursor in mitochondria can be boosted following the intake of 5-aminolevulinic acid (5-ALA), a former molecule in the heme biosynthesis pathway. However, the original hydrophilic 5-ALA toughly cross plasma membranes and showed a poor pharmacokinetic profile.

Designing more lipophilic derivatives such as methyl- and hexyl-5-ALA esters countered the 5-ALA uptake issue. PSI-ALA-Hex, a new phosphatase-sensitive 5-ALA ester performed even better by its reduced acute toxicity and better stability.

In this study, we investigated *in vitro* PpIX production levels in BC cells treated with such 5-ALA derivatives.

Methods

Four BC cell lines were picked to represent main BC tumour subtypes: MCF7 (luminal A), BT-474 (luminal B), SKBR3 (HER2+) and MDA-MB-231 (Triple Negative (TN)). Cells were grown to confluence in 96-well clear bottom black plates and subsequently incubated in the dark with hexylaminolevulinate (HAL) or PSI-ALA-Hex. PpIX production was assessed by fluorescence measurement with a Safire plate-reader.

Results & Discussion

In MCF7, BT-474 and SKBR3 cell lines, PSI-ALA-Hex treatment led to equivalent PpIX production levels, regardless of concentration (from 0.033mM to 1mM), while HAL treatment resulted in large discrepancies. In addition, PSI-ALA-Hex caused similar or higher fluorescence production. Thus, PSI-ALA-Hex could be used at low concentration in photodiagnosis while limiting acute toxicity.

Surprisingly, workable fluorescence in MDA-MB-231, a TNBC cell line, was only reached at 0.33mM and 1mM PSI-ALA-Hex. However, HAL treatment showed a sharp dose-dependent responsiveness and much higher PpIX production level (up to 3-fold for 1mM at 12h). Even though HAL proved to effectively induce PpIX production in MDA-MB-231 at higher concentration than usual, it could be employed as a therapeutic tool in PDT. This is all the more interesting given that TNBC display the most aggressiveness and the worst prognosis among breast cancers.

Conclusion

PSI-ALA-Hex, a new 5-ALA derivative, induces high concentrations of PpIX in all breast cancer cell lines regardless of their hormone receptor status, revealing it as a very promising tool for diagnosis of breast cancer.

Conflicts of Interest

The authors declare no conflict of interest.

> P029. Poster

Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

HOW DOES SUNSCREEN APPLICATION AFFECT EXPOSURE DOSE DURING DAYLIGHT PHOTODYNAMIC THERAPY?

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Daylight photodynamic therapy (dPDT) is increasingly used as an effective, well tolerated and convenient treatment for chronic photodamage (actinic keratoses, (AK)). During dPDT, sunscreen is applied prior to the photosensitiser pro-drug and subsequent exposure to daylight for 2 hours. Sunscreen is an important step in the dPDT process in order to minimise further ultraviolet radiation (UVR) exposure of these patients with chronic photodamage and to reduce the risk of UVR-induced skin reddening (sunburn) by these otherwise dPDT therapeutically ineffective UVR wavelengths.

It was shown in one of the earliest publications on dPDT that the spectral transmittance of inorganic sunscreens would not interfere with the spectrum of light necessary to activate the photosensitiser protoporphyrin-IX (PpIX). However, sunscreen formulations have changed over the years, with increasing emphasis on ultraviolet-A (UVA) protection. Thus, the extent to which modern sunscreens with high UVA protection interfere with the delivery of an effective light dose in dPDT is not known.

To investigate further, we measured the spectral transmittance of several commercial and prescribable sunscreens *in vitro*. The resultant PpIX-weighted exposure dose-to-the-skin was then simulated by applying the spectral transmittance of each sunscreen to the PpIX-weighted spectral irradiance for a representative daylight spectrum and 2-hour treatment. The results showed that PpIX dose was reduced significantly, between 38% and 92%, due to light attenuation by sunscreens.

Additionally, conservatory-based dPDT is becoming an attractive option when there is sufficient daylight for treatment, but outdoor conditions are unsuitable. Currently it is advised that an extra 30 minutes should be added to the treatment duration to account for attenuation of daylight by window glass. Curiously, our data showed that the PpIX-effective dose was reduced by a lesser amount through window glass than by any of the sunscreens. This again opens up the debate around comparative dosimetry methods in different PDT modalities, and how important it is to be fully aware of the impact that these diverse contributing factors may have on effective light dose delivery during dPDT.

> P030. Poster

Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

PROTEOMIC ANALYSIS OF 5-ALA INDUCED CELL CHANGES

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Photodynamic therapy (PDT) has been applied for a long time to treat neoplastic and non-neoplastic diseases. The main problem these therapies are facing nowadays is the weak selectivity for target tissues of most photosensitizers. There are many new approaches to confront this drawback, such as new formulations, delivery systems, quenched derivatives, and so on (1).

To find new approaches to this problem, we aimed to better understand which mechanism is responsible of the selective induction of protoporphyrin IX (PPIX) following the administration of 5-aminolevulinic acid (5-ALA). In our study, we incubated a cell line originating from a human bladder cancer (T24) with 5-aminolevulinic acid (5-ALA) and/or succinylacetone (SA) to study its conversion to downstream metabolic sub-products. 5-ALA is mainly metabolized into Protoporphyrin IX (PPIX), while succinylacetone (SA) is an inhibitor of *ALAD* enzyme (responsible from the metabolic transformation ALA to Porphobilinogen). Following this route, the application of 5-ALA to the cells can break the negative feedback loop induced by the 5-ALA conversion into Heme (2). We intend to comprehend the consequences of this downregulation. Therefore, we made an extensive analysis of the proteome of the T24 cells at two time points, 6 h and 24 h. After treatment with 1 mM 5-ALA and/or 1 mM SA, using non-treated cells as a control of the basal proteome, cells were digested with trypsin. Nine fractions were taken from each sample for high pH reverse phase (HPRP), using an acetonitrile gradient from 1 % to 40 %. Lastly, a solution with 2 µg of protein from each studied condition was prepared for Liquid chromatography–mass spectrometry (LS-MS), using an independent acquisition data method (DIA). With this experimental design, we first intended to build a specific library for our samples, with more than 6'000 proteins, and second to analyze the differences between our conditions, comparing them with our own produced library.

As a result, we observed a significant expression change (q-value<0.05) in more than 200 proteins between the studied groups, some of them with a high interest as future targets due to its specific expression. A t-test was used for the statistics analysis, comparing independently two conditions each time. With this first study, we have built the bases for further studies we intend to pursue, such as obtaining a more complex knowledge of the intracellular PDT mechanism and finding new suitable targets to further improve photodynamic therapies.

References:

1. Calixto GM, Bernegossi J, de Freitas LM, Fontana CR, Chorilli M. Nanotechnology-Based Drug Delivery Systems for Photodynamic Therapy of Cancer: A Review. Molecules. 2016;21(3):342.
2. Wachowska M, Muchowicz A, Firczuk M, Gabrysiak M, Winiarska M, Wańczyk M, et al. Aminolevulinic Acid (ALA) as a Prodrug in Photodynamic Therapy of Cancer. Molecules. 2011;16(5):4140-64.

> P031. Poster

Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

HYDROGEN SULFIDE IN THE CONTEXT OF 5-AMINOLEVULINIC ACID BASED PHOTODYNAMIC THERAPY: MODULATION OF THE RESPONSE IN A MICE BREAST TUMOR CELL LINE MODEL

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Introduction

In 5-aminolevulinc acid based-PDT (ALA-PDT), ALA leads to the synthesis of Protoporphyrin IX (PpIX). Hydrogen sulfide (H_2S) is a gas that belongs to the gasotransmitter family (together with nitric oxide and carbon monoxide), which can diffuse through biological membranes and have relevant physiological effects[1]. It is involved in cardiovascular functions, vasodilatation, inflammation, cell cycle and neuromodulation[2]. It was also proposed to have cytoprotective effects[3]. Our aim was to study the effect of H_2S on ALA-PDT in the LM2 cell line.

Materials and Methods

LM2 cell line (mammary adenocarcinoma murine tumor) was employed. NaSH was employed as source of H_2S. The light source consisted in a bank of fluorescent tubes. Cell survival was quantified by the MTT method. The intracellular reduced glutathione (GSH) was determined using the Ellman´s reagent. PpIX was visualized by fluorescence microscopy and ulterior image analysis. The levels of oxidized proteins were quantified by the 2,4-dinitrophenylhydrazine spectrophotometric assay [4]. Intracellular ROS formation after ALA-PDT was estimated employing 2,7- dichlorofluorescein diacetate by fluorescence microscopy. The capacity of the H_2S to scavenge singlet oxygen, was assessed using the Singlet Oxygen Sensor Green probe®.

Results

Cells exposed to ALA-PDT with different concentration of NaSH (0.1-10 mM) exhibited an increased survival to the PDT treatment in a dose- dependent manner. Light doses leading to 50% of cell death 50 (LD_{50}) of the different treatments were calculated.

H_2S was added at different stages of ALA-PDT treatment: i) 24 h before irradiation, ii) co-incubated with 1 mM ALA; iii) during irradiation; iv) post-PDT, and v) the combination of the three former conditions.

Calculated LD_{50}s were as follows: Control in the absence of H_2S: 114 mJ/cm^2; Treatments: i) 340 mJ/cm^2, ii) 304 mJ/cm^2; iii) 116 mJ/cm^2; iv) LD_{50} 152 mJ/cm^2 and v) >350 mJ/cm^2.

Several parameters were related to H_2S abrogation of ALA-PDT response: a) a slight but significant increase in the levels of GSH in cells incubated with 10 mM H_2S (84 ± 1 nmol/10^6 cells) compared to control cells (73 ±4), b) PpIX accumulation from ALA suffered a dose-dependant reduction after H_2S (0.1-10 mM) exposure, c) the levels of oxidized proteins 4 h after ALA-PDT with NaSH (0.1-10 mM) decreased compared to the treatment without H_2S in a dose dependent manner, d) intracellular ROS after ALA-PDT was diminished after NaSH treatment, e) NaSH decreased the levels of singlet oxygen during an in vitro assay in the absence of cells.

Conclusions

These results suggest that the H_2S has a role in modulating the redox state of the cells, and thus decreasing the response to ALA-PDT through different pathways.

References

[1] Trends biochem Sci. 2015 40(11): 687
[2] Chin Med J. 2013 126(7): 1360
[3] J Cell Mol Med 2012 16:896-910
[4] Anal Biochem. 2014 458: 69-71

> P032. Poster

Symposium PDT-1 ALA-based PDT and photodiagnosis (Norbert Lange)

DAYLIGHT PHOTODYNAMIC THERAPY IS AN OPTION FOR TREATMENT AND CANCER PREVENTION IN PATIENS WITH XERODERMA PIGMENTOSUM IN AFRICA

Authors: Montserrat Fernández Guarino[1], Pablo Fernández González[1], Peter Chapa[2], Carla Ravazzano[3], Lara Jaén[4], Luis Rios[1], Durey Kathry[2], Daudi Mavura[2], Jon Masenga[2], Pedro Jaén[1], Heining Grossman[2]
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Introduction

Xeroderma pigmentosum (XP) is a very rare genetic disorder with a DNA repair defect of ultraviolet (UV)-induced damage. Patients with XP usually develop a great number of skin tumours, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), actinic keratosis (AK), atypical moles, melanoma and severe photoaging. Photodynamic therapy (PDT) was first used in XP in 1991[1], and since them, only three cases have been published, all in white skin[2,3]. Laboratory in vitro investigations demonstrated that cancer associated fibroblast (CAF) in XP had a significantly higher response to PDT compared with normal control fibroblast[4]. PDT has been rarely used in black skin, because of the low incidence of BCC, SCC and AK, PDT has been used to treat aisle cases of inflammatory dermatosis. PDT has also the supposed limitation of the penetration of the light in black skin due to pigmentation[5].

Objective

To explore PDT in patients with XP referred to the RTC in Africa and share the experience with the local dermatologist

Material and methods

Patients with XP referred to the RDTC were evaluated for a group of Spanish and African dermatologist, and patients not candidates for surgery were selected for PDT. Topical Methylaminolevulic acid (MAL, Metvix®, Galderma®) and aminolaevulinic nanoemulsion (ALA, Ameluz®, Biofrontera®) were applied to the patients' faces and were left waiting inside de RDTC for two hours. Afterwards, fluorescence of the lesions was assessed and the cream was removed. Clinical and fluorescence photographs were taken. Patients were examined two days later to assess the reaction to PDT and revised three months later.

Results

A total of 13 patients were treated in the whole face, six females and seven males with a medium age of 12,4years (range 2 to 26). All of the patients presented multiple solar lentigo, generalized photodamage and thin AK in the nose and upper lips. Eight of them also had multiple small pigmented BCC suspected by dermoscopy and thick AK in the central face area. Fluoresce images showed a soft green background color in the face and pink-red delimiting the photodamaged areas. Two days after treatment patients exhibit a crusty and scally reaction in the treated area, more severe in the most damaged areas. After one week the reaction cured, with improvement of the treated area and after three months no adverse events were noticed.

Discussion

PDT is effective as a non-surgical treatment of BCC, multiple AK and for cancer prevention. XP is a severe genetic condition, more severe in Africa, because of the high solar exposure and less access to solar protection (clothes, hats, creams or sun glasses). Daylight PDT is feasible using visible light through a window that blocks ultraviolet B and no need an equipment. It is also is painless and possible in children, which are difficult to remain sitting under a lamp. Black skin has more melanin in the basal layer of the epidermis, but the light can penetrate in the upper layers. To our knowledge, this is the first report of daylight PDT in black skin and in XP, further studies are necessary.

Conclusion

PDT is an option for treatment and cancer prevention in patients with XP.

> IL092. Invited Lecture
Symposium PDT-2 Targeted PDT (Sandy MacRobert)

FOLATE-TARGETED PHOTOSENSITIZER FOR THE TREATMENT OF PERITONEAL METASTASIS OF EPITHELIAL OVARIAN CANCER BY PHOTODYNAMIC THERAPY

Authors: Céline Frochot[1], Ludovic Colombeau[1], Philippe Arnoux[1], Albert Moussaron[2], Samir Acherar[2], Martha Baydoun[3], Henri Azaïs[3], Pierre Collinet[3], Serge Mordon[3], Nadira Delhem[4]
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Background and Objective

Ovarian cancer's prognosis remains dire after primary therapy. 60% of women with epithelial ovarian cancer (EOC) considered in remission will develop recurrent disease within two years. Complete macroscopic cytoreductive surgery of peritoneal metastasis is required as it is the main predictive factors to decrease recurrences. Folate Receptor α (FRa) shows promising prospects in targeting ovarian cancer~~ous~~ cells and peritoneal metastasis[1]. Intraperitoneal PhotoDynamic Therapy (PDT) could be an innovative add-on therapy to macroscopic cytoreductive surgery to treat microscopic peritoneal metastasis.

Our goal is to develop a folic acid targeted photosensitizer and to evaluate the selectivity of the compound, the cytotoxicity and phototoxicity *in vitro* and *in vivo*.

Methods

Different photosensitizers coupled to folic acid were synthesized and analyzed (photophysical properties, photostability)[2,3]

In vitro experiments were performed on ovarian tumor lines Ovcar-3 and Skov-3. In vivo, the proof of concept was assessed on Fischer 344 rat first[4,5]

and then SCID mice xenotransplanted by EOC cells, OVCAR3 expressing luciferase were developed. Then, the evolution of peritoneal carcinomatosis was followed by bioluminescence.

Results and Discussion

A series of compounds has been synthesized with success and the photophysical properties and the photostablity of targeted photosensitizers have been studied. The best one has been patented[6]. *In vitro*, SKOV3 and OVCAR3 treated by PDT with this new targeted photosensitizer presented a significant decrease in their cell viability over time whereas no notable changes in the viability of untreated or PS-only or illumination-only tumor cells has been observed. *In vivo*, we proved that the folic acid targeted photosensitizer has a high affinity for FRa receptor and photodynamic activity.

Conclusion and perspectives

Targeted photodynamic therapy with the novel folic acid photosensitizer could be a solution in addition to macroscopic cytoreductive surgery to treat microscopic peritoneal metastasis.

References

1. van Dam GM, *et al.* Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-α targeting: first in-human results. *Nat. Med.*, **17**(10):1315–1319 (2011).
2. Stallivieri A., *et al.* The interest of folic acid in targeted photodynamic therapy, *Curr. Med. Chem.*, **22**(27):3185-3207 (2015).
3. Stallivieri A., *et al.* Folic acid conjugates with photosensitizer for cancer targeting in photodynamic therapy: synthesis and photophysical properties, *Bioorg. Med. Chem.*, **25**:1-10 (2017).
4. Azaïs H, *et al.* Fischer 344 rat: a preclinical model for epithelial ovarian cancer folate-targeted therapy, *Int. J. Gynecol. Cancer*, **25**(7):1194-1200 (2015).
5. Azaïs H, *et al.* Assessment of the specificity of a new folate-targeted photosensitizer for peritoneal metastasis of epithelial ovarian cancer to enable intraperitoneal photodynamic therapy. A preclinical study, *Photodiagnosis Photodyn. Ther.*, **13**:130-138 (2016).
6. WO2019016397 (A1) - Pyropheophorbide conjugate and use thereof in the treatment of cancer and as a fluorescent marker

> IL093. Invited Lecture
Symposium PDT-2 Targeted PDT (Sandy MacRobert)

ANTIBODY-PHOTOSENSITISER CONJUGATES – SYNTHESIS AND PHOTODYNAMIC ACTIVITY

Authors: Ross Boyle[1], Francesca Bryden[1], Antoine Maruani[2], Joao Rodrigues[1], Miffy Cheng[1], Huguette Savoie[1], Andrew Beeby[3], Vijay Chudasama[2]
Presenting Author: Ross Boyle
1) University of Hull 2) University College London 3) University of Durham

Antibody-drug conjugates (ADC), and biologic drugs in general, are of increasing interest, and open up new options for treatment of major diseases, including cancer. The use of photosensitisers as the drug component of ADCs offers a number of advantages: (i) no release mechanism is required for the active species, as the reactive oxygen species (ROS) required for photodynamic action can be generated while the photosensitiser is still bound to the antibody; (ii) many ROS can be generated from a single photosensitiser, thus removing the requirement to load excessive amounts of drug per antibody; (iii) the combination of antibody targeting and localised light delivery can lead to highly focussed delivery of the therapeutic effect, and help to minimise “off-target effects”. Different synthetic strategies for construction of photosensitiser-based ADCs will be discussed and biological activity of some optimised systems presented.

> IL094. Invited Lecture
Symposium PDT-2 Targeted PDT (Sandy MacRobert)

TARGETED NANOPARTICLES FOR CHEMO- AND PHOTO- KILLING OF CANCER DIFFERENTIATED AND STEM CELLS

Authors: Francesca Moret[1], Elisa Gaio[1], Claudia Conte[2], Fabiana Quaglia[2], Elena Reddi[1]
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Introduction
The reformulation of clinically approved drugs in nanoparticles (NPs) appears to offer the opportunity to ameliorate the efficacy of cancer treatments by increasing drug accumulation via the enhanced permeability and retention (EPR) effect. The construction of targeted NPs is still considered a chance for further increasing selective NP accumulation in malignancies. CD44 is a hyaluronic acid (HA) receptor over-expressed by many cells within the tumor and it is also a well-established marker of cancer stem cells (CSCs), a stem-cell like population that has been identified as the driver of tumor initiation, recurrence and metastasis. The eradication of CSCs represents a challenge in cancer research since they are highly resistant to chemotherapeutics. The combination of different treatment modalities, associated to the use of HA-targeted NPs for the delivery of drugs, appears a valuable strategy to kill simultaneously differentiated and stem cancer cells.

Methods
For targeting CD44, we developed HA-covered layer-by-layer NPs carrying Docetaxel (DTX) entrapped in a PLGA core, covered by a layer of polyetylenimmine (PEI) that entraps electrostatically a photosensitizer (PS) (TPPS4 [1] or TPCS2a [2]). The PEI layer was further covered with a layer of HA. The conceived NP was characterized and CD44-mediated uptake was determined in cancer cells with high and low expression of CD44. Combination treatments were carried out in DTX-sensitive and -resistant cells *in vitro* cultured as monolayers or in a 3D arrangement. In particular, the potential of HA-NPs for the targeting and eradication of CSCs was studied in breast cancer mammospheres, a 3D model enriched in CSCs.

Results
Competition experiments pre-incubating the cells with an excess of HA demonstrated that our NPs are mostly taken up by CD44-receptor mediated endocytosis and that NP uptake is clearly dependent on the level of CD44 expression. Combination of DTX-chemotherapy with TPPS4- or TPCS2a-PDT with the drugs co-loaded in a single HA-NP demonstrated significant superior efficacy than the combination of the drugs delivered in standard solvents or in separate NPs. Moreover, therapy using HA-NPs carrying TPCS2a and DTX showed potent synergism both in DTX-sensitive and DTX-resistant cells cultured in 2D or 3D arrangement. The same nanosystem effectively decreased the percentage of CSCs in MDA-MB-231 and MCF-7 mammospheres as showed by FACS analysis of CD44/CD24 population and ALDH stem markers, and significantly reduced the capacity of first generation mammospheres to propagate in the second generation.

Conclusions
Our HA-NPs have demonstrated to be an efficient platform for combining DTX-chemotherapy and PDT for the eradication of both differentiated and CSCs *in vitro* and possess potential for translating our investigations in *in vivo* tumor animal models.

References

[1] Maiolino S. et al., *Nanoscale*; 2015, 7, 5643-5653.
[2] Gaio E. et al., *Mol. Pharmaceutics*; 2018, 15, 4599-4611.

> IL095. Invited Lecture
Symposium PDT-2 Targeted PDT (Sandy MacRobert)

MESOPOROUS SILICA-BASED NANOPARTICLES FOR TWO-PHOTON PHOTODYNAMIIC THERAPY

Authors: Jean-Olivier Durand[CNRS-]
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Periodic Mesoporous Organosilica Nanoparticles (PMON) have attracted much attention the last decade for nanomedicine applications due to their biocompatibility, flexible functionalisation, tunable pore size and diameter. In this work, we describe the synthesis of porphyrin-based organosilica nanoparticles from large octasilylated metalated porphyrin for two-photon-triggered spatiotemporal theranostics. The nanoparticles displayed unique interconnected large cavities of 10 to 80 nm with a porphyrin-based framework with J-aggregation, which endowed them with two-photon sensitivity. The nanoparticle efficiency for intracellular tracking was first demonstrated by the *in vitro* near-infrared imaging of breast cancer cells. After functionalization with aminopropyltriethoxysilane, Two-photon-excited photodynamic therapy (TPE-PDT) in zebrafish and two-photon photochemical internalization in cancer cells of siRNA-loaded porphyrin-based organosilica nanoparticles were performed. Furthermore, siRNA targeting green fluorescent protein complexed with the nanoparticles was delivered *in vivo* in zebrafish embryos which demonstrated the versatility of the nanovectors for biomedical applications

> IL096. Invited Lecture
Symposium PDT-2 Targeted PDT (Sandy MacRobert)

TRANSFORMABLE NANOTHERANOSTICS FOR PRECISION IMAGE-GUIDED PHOTOTHERAPY

Authors: Xiangdong Xue[Unive], Tzu-yin Lin[Unive], Yuanpei Li[Unive]
Presenting Author: Yuanpei Li
1) University of California, Davis

Nanotheranostics with integrated diagnostic and therapeutic functions show exciting potentials towards precision nanomedicine. However, targeted delivery of nanotheranostics is hindered by several biological barriers. Here, we developed a dual size/charge- transformable, “Trojan Horse” nanovehicle (pPhD NP) for delivery of ultra-small, full active pharmaceutical ingredients (API) nanotheranostics with integrated dual-modal imaging and tri-modal therapeutic functions. pPhD NPs exhibited ideal size (79 nm) and surface charge (12 mV) for drug transportation. In tumour micro-environment, the pPhD NPs responsively transformed to full API nanotheranostics with ultra-small size (~4 nm) and higher surface charge (35 mV), which dramatically facilitated the tumour penetration and cell internalization. pPhD NPs enabled “visualization” of the biodistribution by near-infrared fluorescence imaging, tumour accumulation and therapeutic effect by magnetic resonance imaging. Moreover, the synergistic trimodality therapy (photothermal-, photodynamic- and chemo-therapies) achieved a 100% complete cure rate on both subcutaneous and orthotopic oral cancer models. This nanoplatform with powerful delivery efficiency and versatile theranostic functions shows enormous potentials to improve cancer diagnosis and therapy.

> IL097. Invited Lecture
Symposium PDT-2 Targeted PDT (Sandy MacRobert)

LIGHT-CONTROLLED DELIVERY OF CANCER IMMUNOTHERAPEUTICS

Authors: Judith Wong[1], Monika Håkerud[1], Anne Grete Nedberg[1], Victoria Edwards[1,2], Kristian Berg[1], Anette Weyergang[1], Anders Høgset[2], Pål Kristian Selbo[1]
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Cancer immunotherapeutics including immunotoxins and peptide-based cancer vaccines are taken up into cells by means of endocytosis and are subsequently sequestered and degraded in endosomes and lysosomes. The outcome of this resistance mechanism is lower anti-cancer activity of immunotoxins and poor CD8+ T-cell responses after peptide-based therapeutic cancer vaccines. Thus, there is a need for better intracellular delivery methods which can improve the endosomal escape of immunotherapeutics.

Photochemical internalization (PCI) is an intracellular drug delivery method based on light-induced ROS-generation and a subsequent membrane-disruption of endosomes and lysosomes, leading to cytosolic release of the entrapped drugs of interest. PCI is currently under evaluation in to clinical trials. The overall aim of our project is to develop and explore PCI as a rational strategy to enhance intracellular release and efficacy of (1) immunotoxins targeting cancer stem cells (CSCs) and (2) therapeutic cancer vaccines.

Here we will present data demonstrating fimaporfin ($TPCS_{2a}$)-based PCI-targeting of CSC markers such as CD133, CD44, CSPG4, EpCAM and CD105 (Endoglin). In addition, cancer cells over-expressing stem cell markers important for detoxification such as ABCG2 (BCRP/CD338), ABCB1 (P-gp/MDR1) and ALDH are highly sensitive to photochemical treatment using PCI photosensitizers. Finally, we provide mechanistic evidence showing that PCI strongly enhance MHC class I presentation of peptide vaccine antigens important to mount robust CD8+ specific antitumor responses.

> OC034. Oral Communication
Symposium PDT-2 Targeted PDT (Sandy MacRobert)

SELECTIVE TARGETING AND PHOTODYNAMIC THERAPY OF NON-SMALL CELL LUNG CANCER WITH PEPTIDE CONJUGATED PHTHALOCYANINE GOLD NANOCARRIERS

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This work uses peptides to target PEGylated gold nanoparticles (AuNPs) carrying a zinc phthalocyanine photosensitizer (C11Pc) towards non-small cell lung cancer. Peptides are an attractive targeting moiety as they have highly selective binding towards target receptors whilst presenting a higher tolerance to heat and solvents over antibodies, and they are relatively cheap to synthesise. This tolerance allows for a tighter control over the concentration of targeting ligand on the surface of nanocarriers. While peptides exhibit these obvious advantages, they are rarely utilised, with much of the literature focusing on antibodies for targeting.[1,2] The developed peptide-C11Pc-PEG-AuNPs display selectivity with nanomolar potency upon irradiation, along with minimal dark toxicity; at 200 nM a cell viability of 7 % is observed for irradiated cells, with cell viability above 90 % for non-irradiated cells.

The addition of antioxidants and protease cleavable sequences to these peptides allows for a secondary targeting effect, with these antioxidants able to 'switch off' the photodynamic effect until they are cleaved by proteases overexpressed in cancer cells.

This presentation will focus on the use of peptides to deliver nanocarriers with a double targeting effect to non-small cell lung cancer, discussing the advantages of these systems and displaying their photodynamic ability.

References

1. Obaid, G. et al., *Photochem. Photobiol. Sci.*, **2015**, 14, 737-747
2. Garcia-Calavia, P. et al., *Photochem. Photobiol. Sci.*, **2018**, 17, 281-289

> OC035. Oral Communication
Symposium PDT-2 Targeted PDT (Sandy MacRobert)

NANOBODY-TARGETED PHOTODYNAMIC THERAPY SELECTIVELY KILLS VIRAL GPCR-EXPRESSING GLIOBLASTOMA CELLS

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Introduction
To improve the limited selectivity of conventional PDT, we have introduced an alternative approach for targeted PDT by conjugating photosensitizers to nanobodies (Nbs). This approach has been shown to efficiently and selectively induce toxicity to cells overexpressing the target of interest such as EGFR and C-Met (1,2). Glioblastoma multiforme (GBM) is the most common and most aggressive type of primary brain tumor. The human cytomegalovirus (HCMV) encodes four G protein-coupled receptors (GPCRs), among which US28. US28 is one of the viral chemokine receptors which has been detected in GBM patients and is believed to play a role in oncogenic processes, including the progression of GBM (3). In this study, we evaluated the effect of Nb targeted PDT on US28 expressing cells in 2D and 3D cultures.

Methods
The Nb selectively binding US28 was conjugated to the traceable photosensitizer IRDye700DX maleimide. After characterization of the conjugate, phototoxicity was evaluated on US28 positive and negative cells, cultured in 2D and 3D with 5 mW/cm^2 fluence rate for a total light dose of 10 J/cm^2. The selectivity of the conjugate was investigated in co-culture experiments with US28 expressing and non-expressing cells, using Propidium iodide and Calcein AM staining for dead and live cells, respectively.

Results
The degree of conjugation of Nb-PS was 0.7, with less than 2% of free PS. After PS conjugation, the binding affinity (k_D) on US28 positive cells remained in low nanomolar range (3.1 ± 0.1 nM). Importantly, Nb-targeted PDT selectively and effectively induced cell death in US28 positive cells with nanomolar potency (EC_{50} = 1.1 ± 0.2 nM in 2D and 4.1 ± 1.6 nM in 3D), while it did not cause toxicity in US28 negative cells.

Conclusions
By conjugating the PS to a novel US28-targeting nanobody, we selectively killed US28-expressing cells in 2D and 3D cultures. This study shows for the first time the potential of GPCR-targeting Nbs in targeted PDT as a new potential treatment for HCMV-positive tumors.

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Conflicts of interest
the authors have no conflict of interest to declare

References

1. PBAA. Van Driel, MC. Boonstra, et al., J Control Release, 229 (2016) 93-105
2. R. Heukers, V. Mashayekhi, et al., Antibodies, 8 (2019) 26
3. R. Heukers, T. Shu Fan, et al., Oncogene, 37 (2018) 4110-4121

> OC036. Oral Communication

Symposium PDT-2 Targeted PDT (Sandy MacRobert)

MOLECULAR TARGETED PHOTONANOMEDICINES FOR CANCER: A PATH FROM MODULAR NANOCHEMISTRY TO IN VIVO SPECIFICITY OF DELIVERY AND DESTRUCTION

Authors: Girgis Obaid[1], Shazia Bano[1], Kimberley Samkoe[2], Kenneth Tichauer[3], Srivalleesha Mallidi[4], Diane Simeone[5], Brian Pogue[2], Tayyaba Hasan[1, 6]
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Molecular targeted photonanomedicines (mTarg-PNMs) are highly versatile nanoplatforms for the delivery of multiple synergistic anticancer agents to tumors cells that overexpress specific receptors. They offer unique capabilities of combining multimodal treatment modalities within a single construct, with synchronized delivery to tumors. Furthermore, light-activatable features of photonanomedicines provide exceptional control over induction of photodynamic therapy and photo-triggered release of secondary agents.[1-2]However, during light activation of photonanomedicines in tumors at sensitive anatomical sites such as the pancreas and the brain, toxicity of healthy tissue becomes a limiting factor when moving towards expansive illumination protocols with curative intent.[3]Molecular specificity therefore becomes critical. However, receptor specificity of large nanoconstructs, such as mTarg-PNMs is the focal point of heated debate, as nanomedicine delivery to tumors is heavily influenced by the enhanced permeability and retention effect.[4]

In this study we use a detailed modular nanochemistry approach to fabricate EGFR-specific mTarg-PNMs that co-deliver the approved photosensitizer benzoporphyrin derivative with irinotecan, gemcitabine or 5-fluorouracil chemotherapy to pancreatic cancer organoids and orthotopic *in vivo* tumors.We leverage quantitative molecular imaging to measure the true *in vivo*receptor specificity of our mTarg-PNMs and show them to exhibit optimal EGFR specificity. In an *in vivo* model of pancreatic cancer, we show that mTarg-PNMs induce substantial pancreatic tumor necrosis in a receptor-specific manner, and modulate the collagen content in desmoplastic models that contain patient-derived pancreatic cancer-associated fibroblasts. Critically, the mTarg-PNMs are capable of inducing complete tumor regression, when de-escalating the dose of chemotherapy 20-fold, offering the potential for substantially lower patient toxicities whilst still achieving tumor control.

We thus present an exemplar path towards optimally effective mTarg-PNMs, which incorporates detailed modular nanochemistry and quantifiable *in vivo* specificity, addressing a controversy and providing robust new avenues for molecular targeted phototherapies.

The authors declare that there are no conflicts of interest.

References

1. Luo, D.et al. Lovell, J. F., Doxorubicin encapsulated in stealth liposomes conferred with light-triggered drug release. *Biomaterials***2016,***75*, 193-202.
2. Spring, B. Q. et al. A photoactivable multi-inhibitor nanoliposome for tumour control and simultaneous inhibition of treatment escape pathways. *Nat. Nanotechnol.* **2016,***11*(4), 378-87.
3. Bown, S. G. et al. Photodynamic therapy for cancer of the pancreas. *Gut* **2002,***50*(4), 549-57.
4. Wilhelm, S. et al. Analysis of nanoparticle delivery to tumours. *Nature Reviews Materials* **2016,***1*, 16014.

> P033. Poster

Symposium PDT-2 Targeted PDT (Sandy MacRobert)

EXPLOITING BIOSYNTHETIC GOLD NANOPARTICLES FOR IMPROVING THE AQUEOUS SOLUBILITY OF METAL-FREE PHTHALOCYANINE AS BIOCOMPATIBLE PDT AGENT

Authors: Shaimaa Alexeree[1], Mahmoud Sliem[1], Ragaa EL-Balshy[2], Rehab Amin[1], Mohamed A.Harith[1]
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Background and objectives

Increasing the limit of dispersion of metal-free phthalocyanine (H_2Pc) in an aqueous mediumusing biosynthetic gold nanoparticles for photodynamic therapy (PDT) is investigated. To the best of our knowledge, there isn\'t any study about the unmodified metal-free phthalocyanine (H_2Pc) or even the direct link of H_2Pc to the surface of a nanocarrier and formation of nanoconjugation. In an attempt to overcome the present limitations of PDT in terms of a need for a vehicle to deliver the drug to the tumor tissue and reduce the toxicity of the phthalocyanine derivatives, we present the current work about the successful conjugation between Au NPs and the hydrophobic unmodified H_2Pc with subsequent dispersion of H_2Pc in aqueous medium.

Materials and methods

Gold nanoparticles (Au NPs) are biosynthesized in one step using Potatoes (Solanumtuberosum) extract. The metal-free phthalocyanine is conjugated to the surface of the gold nanoparticles in a side to side regime through the secondary amine groups of H_2Pc. Characterization occurred by UV/VIS spectrophotometry, Fourier transformer infrared spectroscopy (FTIR), and transmission electron microscopy (TEM).

Result and Discussion

The results showed that the biosynthetic Au NPs as well as Pc-Au nanoconjugates have no effect on buffalo epithelial cells viability, which indicating their biocompatibility contrary to the chemically synthesized Au NPs. This work will open the door, for the first time, for using H_2Pc suspended in water for PDT and other phototherapeutic applications.

Conclusion

The present work presents for the first time a new approach for a new generation of hydrophobic photosynthesizers to be utilized in aqueous media.

References

[1] Ormond A., Freeman H. **"Dye Sensitizers for Photodynamic Therapy"**. Materials, 6(3); (2013) 817–840.

> P034. Poster

Symposium PDT-2 Targeted PDT (Sandy MacRobert)

PORPHYRIN-BASED NANOPARTICLES AS A TARGETING VECTOR FOR CANCER THERAPY

Authors: Sofía Domínguez-Gil[1], Christophe Nguyen[2], Vincent Sol[3], Vincent Chaleix[3], Nadir Bettache[2], Magali Gary-Bobo[2], Jochen Roessler[4], Oleg Melnyk[5], Laurence Raehm[1], Jean-Olivier Durand[1], Frédérique Cunin[1]
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Porphyrin-based organosilica nanoparticles (PMOsPOR) as vectors in nanomedicine are getting increasing importance nowadays.[1,2] Among other types of nanoparticles, they seem to be a good option for a wide range of applications due to their biocompatibility and potential biodegradation.[3] PMOsPOR are obtained through the sol-gel condensation in mild conditions using a large octasilylated metallated porphyrin precursor.[4] These nanoparticles present very different characteristics since no silica source for their synthesis is used. These spherical nanoparticles having a diameter nearby 100-300 nm, show porosities ranging from 4-80 nm. The FTIR spectrum presents both characteristic bands of porphyrin moieties but also of siloxane networks.

This work aims to synthesize a suitable vector for cancer therapy combining PMOsPOR and a conotoxin peptide analogue. This system could be capable of targeting acetylcholine receptors, a specific marker of tumors such as rhabdomyosarcoma. To carry out this strategy, it is necessary to modify the surface of these nanoparticles by introducing a coupling agent. The yield of coupling is quantified by UV spectroscopy analyzing the supernatant of the reaction.[5] Subsequent PMOsPOR anchoring of conotoxin peptide will allow to perform imaging, two-photon excited photodynamic therapy (TPE-PDT) and TPE-induced siRNA delivery of rhabdomyosarcoma.

References

(1) Croissant, J.; Cattoën, X.; Man, M. W. C.; Gallud, A.; Raehm, L.; Trens, P.; Maynadier, M.; Durand, J.-O. *Adv. Mater.* **2014**, *26* (35), 6174–6180.
(2) Croissant, J. G.; Mauriello-Jimenez, C.; Maynadier, M.; Cattoën, X.; Wong Chi Man, M.; Raehm, L.; Mongin, O.; Blanchard-Desce, M.; Garcia, M.; Gary-Bobo, M.; et al. *Chem. Commun.* **2015**, *51* (61), 12324–12327.
(3) Chen, Y.; Meng, Q.; Wu, M.; Wang, S.; Xu, P.; Chen, H.; Li, Y.; Zhang, L.; Wang, L.; Shi, J. *J. Am. Chem. Soc.* **2014**, *136* (46), 16326–16334.
(4) Mauriello Jimenez, C.; Aggad, D.; Croissant, J. G.; Tresfield, K.; Laurencin, D.; Berthomieu, D.; Cubedo, N.; Rossel, M.; Alsaiari, S.; Anjum, D. H.; et al. *Adv. Funct. Mater.* **2018**, *28* (21), 1800235.
(5) Ollivier, N.; Desmet, R.; Drobecq, H.; Blanpain, A.; Boll, E.; Leclercq, B.; Mougel, A.; Vicogne, J.; Melnyk, O. *Chem. Sci.* **2017**, *8* (8), 5362–5370.

> P035. Poster

Symposium PDT-2 Targeted PDT (Sandy MacRobert)

COVALENTLY CROSS-LINKED TETRAFUNCTIONALIZED m-THPC CHITOSAN HYDROGELS AS DELIVERY PLATFORMS

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Photodynamic therapy (PDT) is a modified anti-cancer treatment method, which uses the combined effect of a photosensitizing drug (as pro-drug activating agent), light, and oxygen to cause selective damage to target tissue.[1] The second generation photosensitizer (PS) 5,10,15,20-tetrakis (*m*-hydroxyphenyl) chlorin (*m*-THPC) is a widely characterized, clinically tested, and commercially available drug.[2] Furthermore, in order to develop advanced treatment modalities there is a need for improved drug delivery platforms. Hydrogels, which have been investigated as effective and site-specific drug delivery systems, can prevent PSs aggregation and offer significant potential as carriers due to their ability to swell in aqueous media.[3]

In the present work, *m*-THPC was used as a starting point for new synthetic strategies to obtain a library of compounds aimed at overcoming this PS's limitations while maintaining the photophysical and clinical properties of *m*-THPC. Substitution, esterification and Sonogashira coupling reactions were employed to modify the *m*-THPC skeleton using a variety of halogen or carboxylic group containing moieties. These novel derivatives are expected to maintain efficient $^1O_2$ production and high biological activity. Chitosan[4] presents suitable, biodegradable and antimicrobial material properties to generate hydrogels. Thus, tetrafunctionalization of *m*-THPC introduces aldehyde or carboxylic acid functionalities and provides a suitable synthetic handle for covalent cross-linking PS with the polymer backbone.

References:

1. T. J. Dougherty, C. J. Gomer, B. W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, Q. Peng, *J. Nat. Cancer Inst.* **1998**, *90*, 889–905.
2. M. O. Senge, J. C. Brandt, *Photochem.Photobiol.* **2011**, *87*, 1240–1296.
3. S. Belali, H. Savoi, J. M. O'Brien, A. A. Cafolla, B. O'Connell, A. R. Karimi, R. W. Boyle, M. O. Senge, *Biomacromolecules* **2018**, *19*, 1592–1601.
4. J. Nilsen-Nygaard, S. P. Strand, K. M. Vårum, K. I. Draget, C. T. Nordgård, *Polymers* **2015**, *7*, 552–579.

> P036. Poster

Symposium PDT-2 Targeted PDT (Sandy MacRobert)

BIOCONJUGATABLE, LONG WAVELENGTH ABSORBING GEM-DIMETHYL CHLORINS: THE CONTINUAL SEARCH FOR SUPERIOR PHOTOSENSITIZERS

Authors: Harry Sample[1], Zoi Melissari[1], Lígia Gomes-da-Silva[2], Mathias Senge[1,3]
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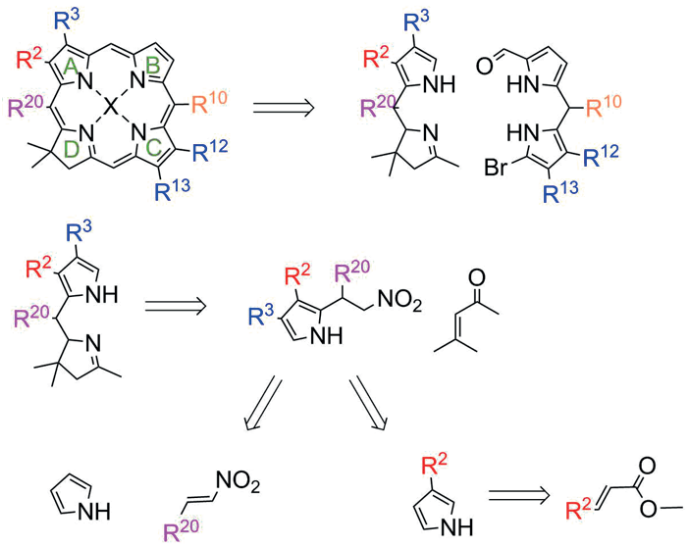
Photodynamic Therapy (PDT) is a non-invasive treatment that relies upon a photosensitizer (PS), being excited by light of a suitable wavelength such that the PS can then react with molecular oxygen. This forms a variety of cytotoxic reactive oxygen species (ROS) which can kill cancerous cells. Next to porphyrins, chlorins have long been considered good PSs and PDT drug candidates. Chlorins currently in clinical practise (e.g., Temoporfin and Visudyne) are either limited in stability towards oxidants,[1] or offer only limited possibilities for further synthetic elaboration.[2] Thus, more generally applicable synthetic strategies to stable chlorin PSs are needed. Ever since the first report of geminal dialkyl chlorins,[3] the doorway to stable synthetic chlorins has been wide open. This has led the use of *gem*-dimethyl chlorins for catalysis, generation of Near Infra-red emissive dyads, and aided the synthesis of naturally occurring hydroporphyrins.[4]

Herein we report the synthesis of a variety of novel *gem*-dimethyl chlorins bearing substituents on four of the six pyrrolic *β*-positions, and two of the meso-positions on the chlorin macrocycle, through the use of the Lindsey [2+2] type chlorin synthesis.[2] Utilization of the van-Leusen pyrrole synthesis yields control of substitution on the A ring and generation of novel *β*-nitrostyrenes yields functionality at the 20 position. All of these functionalities (1-naphthyl, and *p*-C_6H_4-X where X = H, Br, CN, NO_2, SO_3Me, $P(O)(OEt)_2$) yield desirable properties such as water solubility, the possibility for π-extension, and bioconjugation. The obtained water-soluble chlorins will be subject to *in vitro* cell tests against a variety of cancer lines, along with studying the intracellular localization of the various PSs.

References

[1] J. S. Lindsey, *Chem. Rev.*, **2015**, *115*, 6534–6620.
[2] M. O. Senge, *Photodiagn. Photodyn. Ther.*, **2012**, *9*, 170–179.
[3] J. P. Strachan, D. F. O'Shea, T. Balasubramanian, J. S. Lindsey, *J. Org. Chem.*, **2000**, *65*, 3160–3172.
[4] Y. Liu, S. Zhang, J. S. Lindsey, *Nat. Prod. Rep.*, **2018**, *35*, 879–901.



> P037. Poster

Symposium PDT-2 Targeted PDT (Sandy MacRobert)

DEREGULATION OF Wnt/β-CATENIN SIGNALING PATHWAY IN SQUAMOUS CARCINOMA CELLS SUBJECTED TO REPEATED CYCLES OF PHOTODYNAMIC THERAPY

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Squamous Cell Carcinoma (SCC) is the second most frequent type of skin cancer among the population. Within the clinically approved treatments, photodynamic therapy (PDT) is an extended non-invasive therapeutic modality. However, after PDT resistant cells may appear. PDT-resistance mechanisms have been barely studied, especially in MAL-PDT. In this study, SCC-13 human line was used as model of SCC. This cell line, called parental (P), was subjected to 10 PDT cycles to obtain resistant cells (10G) that were inoculated in immunosuppressed mice; the induced tumors were sub-cultured by explants and a cell population called 10GT was obtained. In order to determine the factors responsible of PDT resistance and their cellular consequences we analyzed differences between the studied cells referring to therapy sensitivity, proliferation, spheroid formation and genomic variation through a CGH array. Interestingly, 10GT line was more resistant to PDT than 10G cells, indicating a possible tumor reselection of resistant cells in the animals. The number of colonies was significantly higher in 10G and 10GT than in P line, appreciating also relevant differences in their size between cell lines. Accordingly, the number of formed spheroids was higher in both resistant cell lines. Last, CGH analysis revealed alterations in multiple genes, including CCND1 and LRP5, both elements of Wnt/β-catenin signaling pathway. The expression of selected genes of interest that participate in the Wnt/β-catenin pathway was confirmed by RT-PCR, Western blot and immunofluorescence. Altogether, these results evidence that deregulation of Wnt/β-catenin signaling pathway seems to be an important step during PDT resistance acquisition.

> P038. Poster

Symposium PDT-2 Targeted PDT (Sandy MacRobert)

PHOTODYNAMIC TREATMENT OF MELANOMA USING AZA-DIPYRROMETHENES AS PHOTOSENSITISERS

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Aza-bodipys are versatile organic dyes endowed of unique properties, such a strong absorption and fluorescence emission at long wavelengths, but also a great photostability and ability to generate singlet oxygen. These properties can be easily modulated through the synthetic introduction of different functional groups in their backbone, which makes them especially attractive for several applications, including in the photovoltaic and optoelectronic field, sensing, bioimaging, photodynamic therapy (PDT) and also as theragnostic agents [1,2,3].

Aza-dipyrromethenes are the synthetic precursors of the azabodipy dyes (BF_2 chelates). However, these intermediates by itself remain neglected and, as far as we know, nothing has yet been described about the possible applications of these derivatives.

In this communication, we report the ability of these compounds to be used as photosensitisers in the photodynamic therapy of cancer. For this purpose, we synthesised four azadipyrromethenes bearing different substituents (with donor or acceptor character) in 3,5-diphenyl rings. The influence of these substituents on their photophysical properties was evaluated. The activity of these compounds against a resistant melanoma cell line (B16F10) was evaluated and the results will be presented and discussed.

Acknowledgements

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. L. Jiao, Y. Wu, S. Wang, X. Hu, P. Zhang, C. Yu, K. Cong, Q. Meng, E. Hao and M. G. H. Vicente, *J. Org. Chem.*, 2014, **79**, 1830–1835.
2. A. Kamkaew, S. H. Lim, H. B. Lee, L. V. Kiew, L. Y. Chung and K. Burgess, *Chem. Soc. Re.v*, 2013, **42**, 77–88.
3. Y. Ge and D. F. O'Shea, *Chem Soc Rev*, 2016, **45**, 3846–3864.

> P039. Poster

Symposium PDT-2 Targeted PDT (Sandy MacRobert)

CHLORIN AND ISOBACTERIOCHLORIN DERIVATIVES AS POTENTIAL PHOTOSENSITIZING AGENTS FOR PHOTODYNAMIC THERAPY

Authors: Mariana Q. Mesquita[1,2], M. Graça P. M. S. Neves[1], Margarida Fardilha[2], M. Amparo F. Faustino[1]
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Photodynamic therapy (PDT) is a promising methodology that can be applied in the treatment of several types of cancer. This therapy relies on the use of a non-toxic photosensitizer (PS) that is selectively activated by light to provoke cell death through reactive oxygen species generation (1). Porphyrins and analogues are the most extensively studied PSs and some of them have already been approved for clinical use. Under this context, some chlorin and isobacteriochlorin derivatives have already been investigated as PSs in PDT with promising results (2-4). In fact, chlorins and isobacteriochlorins are distinguished from the parent porphyrins by the presence of reduced peripheral double bonds. This change in symmetry leads to greater absorption in the red region of the visible spectrum, allowing them to cause deeper tissue photodamage than porphyrins (5). Bearing this in mind, we have prepared chlorin and isobacteriochlorin derivatives using as template 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin and have evaluated their efficacy as PS against one prostate cancer cell line (PC-3). Here, we will describe and discuss the synthetic strategy giving access to the PSs, their spectroscopic and photophysical properties, as well as their photodynamic efficacy against prostate cancer cells.

Acknowledgments

Thanks are due to the University of Aveiro and FCT/MCT for the financial support of the QOPNA research Unit (FCT UID/QUI/00062/2019) and Institute for Biomedicine – iBiMED (UID/BIM/04501/2013 and POCI-01-0145-FEDER-007628) through national funds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement, and to the Portuguese NMR Network. MM thanks FCT for her doctoral grant (SFRH/BD/112517/2015).

Conflicts of Interests

The authors declare no conflict of interest.

References

1) Mesquita, M. Q., Dias, C. J., Gamelas, S., Fardilha, M., Neves, M. G. P. M. S., Faustino, M. A. F. *An. Acad. Bras. Ciênc.*2018, 90, 1101-1130.
2) Singh, S., Aggarwal, A., Thompson, S., Tomé, J. P. C., Zhu, X., Samaroo, D., Vinodu, M., Gao, R., Drain, C. M. *Bioconjug Chem.*2011, 21, 2136-2146.
3) Aggarwal, A., Thompson, S., Singh, S., Newton, B., Moore, A., Gao, R., Gu, X., Mukherjee, S., Drain, C. M. *Photochem. Photobiol.*2014, 90, 419-430.
4) Cui, X., Li, Y., Li, Y., Qiu B., Duan, Q. *Dyes Pigm.*2019, 164, 237-243.
5) Lee, S-R., Kim Y-J. *Nanomaterials (Basel)*. 2018, 8, 445.

> P040. Poster

Symposium PDT-2 Targeted PDT (Sandy MacRobert)

SITE-SPECIFIC BIOORTHOGONAL LIGATION AND ACTIVATION OF BODIPY-BASED PHOTOSENSITIZER FOR TARGETED PHOTODYNAMIC THERAPY

Authors: Xuejiao Guo, Pui Chi Lo
Presenting Author: Xuejiao Guo
1) Department of Biomedical Science, City University of Hong Kong, Hong Kong

Photodynamic therapy (PDT) is a promising therapeutic modality for cancer.[1] However, the low selectivity of photosensitizers between normal and tumor tissues severely limits the clinical use of PDT.[2] The traditional method for targeted PDT requires direct conjugation of the tumor-targeting moiety to the photosensitizers, but the conjugation may affect the binding affinity and biocompatibility of the targeting ligands, limiting the targeting ability towards tumor.[3] Bioorthogonal chemistry is emerging as an advanced technique for tumor-targeted delivery, which could avoid the steric hindrance effects through the separate administration of targeting domain and large therapeutic agents.[4]

In this presentation, we report the chemical design, synthesis, *in vitro* and *in vivo* biological activities of a novel boron dipyrromethene (BODIPY)-based photosensitizer substituted with two biorthogonal function groups. The trans-cyclooctene (TCO)-modified epidermal growth factor receptor binding peptide (GE11-TCO) or tetraacetyl-N-azidoacetylmannosamine (Ac4ManNAz) is used for the pre-targeting. This BODIPY photosensitizer can react with the TCO or azide groups expressed on tumor cell surface through bioorthogonal reactions. We found that the cellular uptake and photocytotoxicity of this photosensitizer were enhanced against A431 cells when using pre-targeting method. In particular, this photosensitizer exhibited about 2-fold higher fluorescence signal at the tumor site of nude mice through bioorthogonal reaction. The overall results showed that this bioorthogonal-functionalized BODIPY photosensitizer can serve as a promising therapeutic agent for PDT.

Acknowledgement
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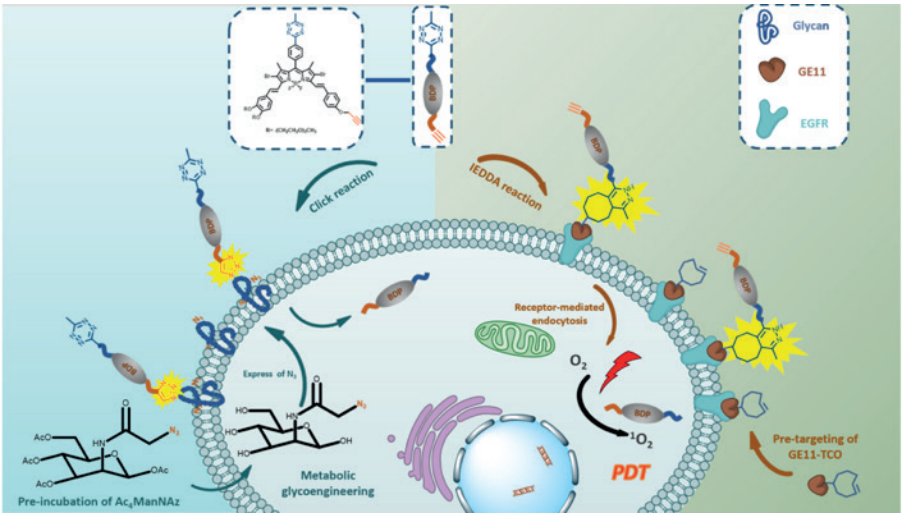
Conflicts of Interest
The authors declare no conflicts of interest.

References

1. D. E. Dolmans, D. Fukumura, R. K. Jain, *Nature reviews cancer*, 2003, 3 (5), 380.
2. M. Ethirajan, Y. Chen, P. Joshi, R. K. Pandey, *Chemical Society* Reviews, 2011, 40 (1), 340-362.
3. J. F. Lovell, T. W. Liu, J. Chen, G. Zheng, *Chemical reviews*, 2010, 110 (5), 2839-2857.
4. C. P. Ramil, Q. Lin, *Chemical Communications*, 2013, *49* (94), 11007-11022.



> P041. Poster

Symposium PDT-2 Targeted PDT (Sandy MacRobert)

c(RGDfK) AND ZnTriMPyP-MODIFIED POLYMERIC NANOCARRIERS FOR TUMOR-TARGETED PHOTODYNAMIC THERAPY

Authors: Elena de las Heras[1], Ester Boix-Garriga[1, 2], Montserrat Agut[1], M. Lluïsa Sagristá[3], Ross W. Boyle[4], Norbert Lange[2], Santi Nonell[1]

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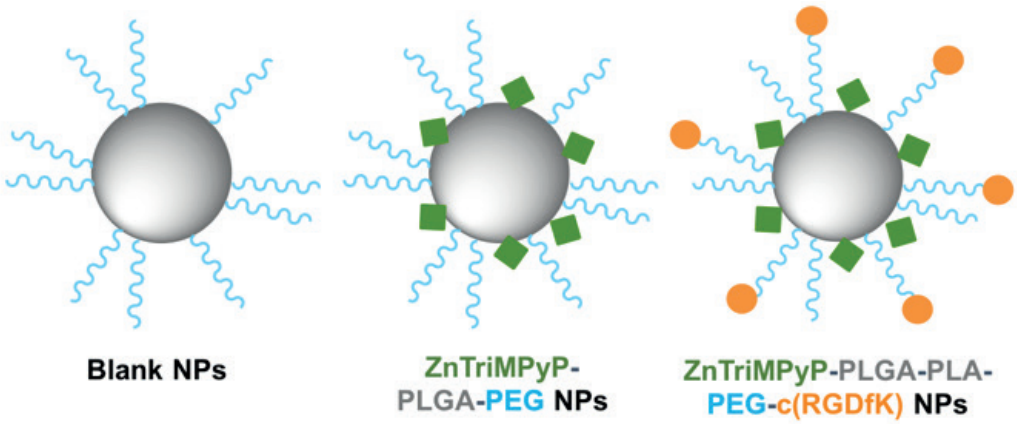
Active targeting strategies are currently being extensively investigated in order to enhance the selectivity of photodynamic therapy. The aim of the present research is to evaluate if the external decoration of nanopolymeric carriers with targeting peptides could add more value to a photosensitizer formulation and increase antitumor therapeutic efficacy and selectivity. For this reason, we have assessed PLGA-PLA-PEG nanoparticles (NPs) covalently attached to a hydrophilic photosensitizer, ZnTriMPyP, and also to c(RGDfK) peptides, in order to target $\alpha_v\beta_3$ integrin expressing cells. To achieve this goal, the synthetic conjugation of ZnTriMPyP and c(RGDfK) peptide to the polymeric chains has been performed. Three types of NPs have been prepared by nanoprecipitation and characterized physicochemically and photophysically: blank, ZnTriMPyP-PLGA-PEG and ZnTriMPyP-PLGA-PLA-PEG-c(RGDfK) NPs. In this regard, it has been demonstrated that NPs conjugated with ZnTriMPyP generate singlet oxygen, confirming their suitable properties as photosensitizers. Furthermore, the biological activity of the prepared NPs has been studied in high expressing $\alpha_v\beta_3$ integrin cancer cells (U-87 MG) and in remarkably low expressing tumor cells (HeLa) irradiating with blue light. *In vitro* phototoxicity investigation indicates that the novel nanocarrier ZnTriMPyP-PLGA-PLA-PEG-c(RGDfK) is a potential photo-anti-tumoral agent. It is effective at nanomolar concentrations, devoid of dark toxicity and successfully targets $\alpha_v\beta_3$ integrin expressing cells. However, non-specific internalization due to the enhanced permeation and retention effect is a major uptake channel in both cell lines.

Acknowledgements

This work has been supported by grant CTQ2016-78454-C2-1-R from the Spanish Ministerio de Economía y Competitividad.

References

Boix-Garriga, E. *et al.*, Poly-(D,L-Lactide-Co-Glycolide) Nanoparticles with Covalently-Bound Porphyrins for Efficient Singlet Oxygen Photosensitization. *J. Porphyr. Phthalocyanines* **2016**, *20* (08n11), 1306–1318.



> P042. Poster

Symposium PDT-2 Targeted PDT (Sandy MacRobert)

BIOLOGICAL EFFECTS OF PORPHYCENE DERIVATIVES IN PDT TREATMENTS

Authors: Mireia Jordà-Redondo[1 2], Cormac Hally[1 3], Íngrid Nieves[1], M. Lluïsa Sagristá[2], Montserrat Agut[1], Santi Nonell[1]
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Finding photosensitizers with subcellular targets can be an advantage for their phototoxic activity. In this study, we intend to analyze different porphycene derivatives to assess their properties to induce cell death. Porphycenes are photosensitizers which have optimal optical properties for PDT, but with solubility issues in physiological media. Attempts to improve its solubility can go along with the development of conjugates which target specific subcellular localizations.

A total of 4 conjugates are presented. The *in vitro* assays were performed on tumor cells (HeLa) and fungi (*C. albicans*). In HeLa, the conjugates were analyzed with triphenylphosphonium as a lipophilic cation, gentamicin as an antibiotic and an analogous porphycene (butylamine) devoid of a targeting group. The aforementioned conjugates were tested also on *C. albicans*. The biological *in vitro* assays show that these conjugates are able to photoinactivate mammalian cells at submicromolar concentrations, whilst higher concentrations in *C. albicans* were required. Different conjugation moieties with porphycene derivatives may sublocalized to different places within the cell, which could explain the differences in the outcome of the treatment.

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References

Ruiz-González, R.; Acedo, P.; Sánchez-García, D.; Nonell, S.; Cañete, M.; Stockert, J. C.; Villanueva, A. Efficient Induction of Apoptosis in HeLa Cells by a Novel Cationic Porphycene Photosensitizer. *Eur. J. Med. Chem.* **2013**, *63*, 401–414.

Bresolí-Obach, R.; Gispert, I.; Peña, D. G.; Boga, S.; Gulias, Ó.; Agut, M.; Vázquez, M. E.; Nonell, S. Triphenylphosphonium Cation: A Valuable Functional Group for Antimicrobial Photodynamic Therapy. *J. Biophotonics* **2018**, *11* (10).

Gilson, R. C.; Tang, R.; Gautam, K. S.; Grabowska, D.; Achilefu, S. Trafficking of a Single Photosensitizing Molecule to Different Intracellular Organelles Demonstrates Effective Hydroxyl Radical-Mediated Photodynamic Therapy in the Endoplasmic Reticulum. *Bioconjug. Chem.* **2019**, *30*, 1451–1458.

> IL098. Invited Lecture

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

CO-DELIVERY OF PDT PHOTOSENSITIZERS AND CHEMOTHERAPEUTICS BY NANOPARTICLES FAVORS SYNERGIC EFFECTS OF COMBINED TREATMENTS

Authors: Elisa Gaio[1], Francesca Moret[1]
Presenting Author: Elena Reddi
1) Department of Biology, University of Padova

Introduction

The combination of different modalities of treating cancer is considered a valid strategy to ameliorate the disease control and cure. Thus, the combination of chemotherapy and photodynamic therapy (PDT) is being investigated with the aim to reduce unwanted generalized toxic effects caused by chemotherapeutics and photosensitizers (PS). In fact, in the combination, the doses of both drugs can be reduced, and the efficacy increased, with respect to monotherapies, provided that optimal drug ratios yielding synergic interactions are identified [1].

Methods

We have investigated on the combination of chemotherapy and PDT using docetaxel (DTX) and disulphonate tetraphenyl chlorin (TPCS2a) or chlorin e_6 (Ce_6) co-loaded in one nanoparticle (NP) for guaranteeing that the optimal drug ratio was delivered to cancer cells. Layer-by-layer NPs and keratin NPs loaded with DTX in addition to TPCS2a and Ce6, respectively, were used. The effects of the combination were determined in cancer cells (MDA-MB-231, DTX-sensitive and -resistant HeLa) grown in monolayers and in spheroids mimicking avascular tumors.

Results and Discussion

Dose-response curves generated after single treatments of MDA-MB-231 and HeLa cell monolayers indicated the DTX/TPCS2a ratio of 1:35 (w/w) as optimal for combined treatments. The combination index (CI) [2] showed that at this ratio, combined treatments delivering the drugs co-loaded in the same NP gave higher synergism than the co-administration of the free drugs. The difference was particularly important for MDA-MB-231 and DTX-resistant HeLa cells. Surprisingly, the treatments of HeLa cell spheroids with the combination of DTX and TPCS2a/PDT at this drug ratio gave antagonist effects while synergism could be found by co-loading NPs with the DTX/TPCS2a ratio of 1:3. As for keratin NPs, the chemo-Ce6/PDT combination was particularly effective in DTX-resistant HeLa cells and again the drug co-loading in keratin NPs produced synergic interaction between chemotherapy and PDT. In addition, as respect to monotherapies, the combination induced stronger cytotoxicity to spheroids of both DTX-sensitive and -resistant HeLa cells by reducing their volumes up to 50%.

Conclusions

The delivery of PSs and chemotherapeutics co-loaded in one NP increases the chances to obtain a synergic interaction between PDT and chemotherapy. The synergic effect allows a reduction of the drug doses, especially DTX, with respect to monotherapies while preserving efficacy. Our data highlighted also that monolayers can be unsuitable *in vitro* tumor models for determining the optimal drug ratio giving synergism in the combination.

References

[1] Dicko A, Mayer LD, Tardi PG. Expert Opin Drug Deliv. 2010; 7:1329-41; [2] Chou, T. C. Pharmacol. Rev. 2006, 58, 621−681.

> IL099. Invited Lecture

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

PHOTODYNAMIC THERAPY IN THE COMBINED MODALITY SETTING: FLUENCE RATE AS A FACTOR

Authors: Theresa Busch[1]
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Introduction

Photodynamic therapy (PDT) can uniquely contribute to cancer treatment in the combinational setting. In the design of combined modality therapy, it can be useful to consider PDT effect on the tumor microenvironment so as to develop rational approaches that maximize synergies between the individual modalities. As an example, PDT effect on the molecular signature of a tumor may inform potential combinations with complimentary treatment. Through investigations that incorporate clinical through preclinical research, we have considered how the attributes of PDT delivery can suggest choice of combined modality approaches.

Methods

In thoracic PDT for diffuse malignancy of the pleura, light is delivered intraoperatively throughout the thoracic cavity after surgical debulking. Illumination is achieved through the patterned movement of a source throughout the cavity, and we have quantified the deposition of fluence rate as a function of this movement. The fluence rate of light delivery is well known to affect numerous aspects of response to PDT, such as tumor oxygenation and vascular damage. In a murine model of intrathoracic PDT, we have additionally considered how fluence rate may affect PDT-induced survival signaling through the epidermal growth factor receptor (EGFR)[1].

Results

PDT of the thoracic cavity is associated with heterogeneity in tissue-incident fluence rate on both a temporal and spatial basis. During light delivery, most tissues experienced median fluence rates of ~30 – 60 mW/cm^2, but the range in instantaneous fluence rate was from 0 to >300 mW/cm^2. In a murine model of intrathoracic PDT, higher incident fluence rate was associated with greater EGFR activation in residual tumor burden. Higher fluence rate was also less effective in reducing intrathoracic tumor burden.

Conclusions

Clinical investigations of intrathoracic PDT have quantified typical distributions of fluence rate within and between patients during light delivery throughout the cavity. Preclinical studies in a murine model of intrathoracic PDT indicate that PDT-induced survival signaling through EGFR may be affected by incident fluence rate. These data suggest that the efficacy of combined modality approaches that incorporate EGFR inhibitors with PDT may differ as a function of treatment fluence rate. Ongoing studies are assessing combinations of PDT with EGFR inhibition over a range of photosensitizing and illumination conditions.

References

[1]Grossman CE, Carter SL, Czupryna J, Wang L, Putt ME, Busch TM. *Fluence Rate Differences in Photodynamic Therapy Efficacy and Activation of Epidermal Growth Factor Receptor after Treatment of the Tumor-Involved Murine Thoracic Cavity*. Int J Mol Sci. 2016 Jan 14;17(1). pii: E101. doi: 10.3390/ijms17010101. PMC4730343.

> IL100. Invited Lecture

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

EXPLORATION OF ADVANCED PHOTODYNAMIC MOLECULAR BEACONS AND NANOPHOTOSENSITIZING SYSTEMS FOR TARGETED AND ENHANCED PHOTODYNAMIC THERAPY

Authors: Di Gao[1], Xuejiao Guo[1], Ligang Yu[1]
Presenting Author: Pui Chi Lo
1) City University of Hong Kong

Photodynamic therapy (PDT) has been used for the treatment of various cancers, including esophagus, lung, ovarian, and skin. It utilizes a photosensitizer, an appropriate wavelength of light, and molecular oxygen to generate cytotoxic reactive oxygen species (ROS), causing oxidation of cellular components and tumor cell ablation.[1] Much research effort has been devoted to developing advanced photosensitizing systems that can achieve tumor specificity, improved efficacy, and fewer side effects. The emergence of activatable photosensitizers in the past decade has shed light on the direction of further development of more advanced photosensitizers [2]. Moreover, PDT has been investigated to combine with other therapeutic methods to induce different cytotoxic pathways, resulting in enhanced therapeutic efficacy [3]. In this presentation, we report our recent studies of advanced photodynamic molecular beacons and a series of nanophotosensitizing systems combining photosensitizers with doxorubicin, hypoxic cytotoxin tirapazamine, or hypoxia-inducible factor 1 inhibitor acrifavine for targeted and enhanced PDT. The design, preparation, characterization, photophysical properties, in vitro and in vivo biological activities of these photosensitizing systems will be presented.

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References

[1] Dolmans, D. E. J. G. J.; Fukumura, D.; Jain, R. K. Photodynamic Therapy for Cancer. *Nat. Rev. Cancer* **2003**, *3*, 380-387.

[2] Lovell, J. F.; Liu, T. W. B.; Chen, J.; Zheng, G. Activatable Photosensitizers for Imaging and Therapy. *Chem. Rev.* **2010**, *110*, 2839-2857.

[3] Brodin, N. P.; Guha, C.; Tomé, W. A. Photodynamic Therapy and Role in Combined Modality Anticancer Treatment. *Tech. Cancer Res. Treat.* **2015**, *14*, 355-368.

> IL101. Invited Lecture

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

DEVELOPING LIGHT-BASED COMBINATION STRATEGIES TO OVERCOME RESISTANCE MECHANISMS IN PANCREATIC CANCER

Authors: Pilar Acedo[1,2], Pål K. Selbo[3], Patricia Sancho[4], Ángeles Villanueva[5], Sandy MacRobert[2], Stephen P. Pereira[1]
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Introduction

Determining the best combination of therapies for patients with advanced stage cancers is a major goal, however conventional approaches (e.g. chemotherapy) have had only a minor impact on survival rates of the most aggressive types of cancers. Reduced intracellular drug accumulation coupled with multi-drug resistance are among the most common mechanisms of resistance to therapy of solid tumours. Novel therapeutic options such as minimally-invasive light-based strategies may have a potentially important role in overcoming these limitations.[1,2] Since chemotherapy is already established, demonstration of effective combinations with photodynamic therapy (PDT) would have significant research impact together with wider clinical indications.

Methods

In this project, we have designed a combination of photosensitisers targeting different subcellular compartments to improve the efficacy of PDT while minimising toxicity.[1] Moreover, we have also evaluated photochemical internalisation (PCI) as a light-triggered drug delivery technique to enhance the therapeutic effect of different drugs (e.g. gemcitabine, Akt inhibitors) and to overcome the evasion pathways that cause resistance.[3] Treatment efficacies were assessed in both 2D and 3D patient-derived pancreatic tumour models, by different cell viability assays and molecular techniques. The activation of cell death pathways after treatment was evaluated by protein array-based approaches.

Results and Discussion

Our studies indicate that light-based combination strategies can significantly improve treatment outcomes and enhance on-site drug release. We have demonstrated that PDT can exert a synergistic effect with conventional agents such as gemcitabine and attenuate chemotherapy-induced treatment resistance by inactivating key survival signalling pathways. Moreover, our recent studies also confirm that nanotechnology can provide state-of-the-art drug delivery systems for multimodal and targeted cancer therapy.

Conclusions

Our findings demonstrate the potential of PDT-based combination strategies to improve the therapeutic index of anticancer drugs used for treating pancreatic cancer. These studies open a new window for identifying more effective and clinically relevant multimodal approaches to treat highly resistant cancers.

Acknowledgements

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Conflicts of interest

The authors declare no competing financial interest.

References

1. Acedo P *et al.* (2014) Cell Death Dis. doi: 10.1038/cddis.2014.77.
2. Huggett MT *et al.* (2014) Br J Cancer. 110:1698-1704.
3. Acedo P *et al.* (2017) Photodiagnosis Photodyn Ther. doi: 10.1016/j.pdpdt.2017.01.065

> OC037. Oral Communication
Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

GIVING NANOMEDICINE A BOOST: INCREASING TUMOR NANOPARTICLE UPTAKE WITH SUBTHERAPEUTIC PHOTODYNAMIC THERAPY

Authors: Marta Overchuk[1,2], Kara Harmatys[2], Shrey Sindhwani[1], Abdullah Syed[1], Juan Chen[2], Martin Pomper[3], Warren Chan[1], Brian Wilson[2,4], Gang Zheng[2,4]
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Rational
Despite recent advances in nanotechnology for tumor drug delivery, only a small fraction of the injected nanoparticles reaches cancer cells due to the strong uptake by clearance organs as well as barriers within the tumor microenvironment. Few studies have explored subtumoricidal photodynamic therapy (PDT) as a means of enhancing nanomedicine delivery to the solid tumor, but the utility and mechanism of this approach has not been fully realized.

Methods
We employed a low-molecular-weight (<2 kDa) bacteriochlorophyll-based photosensitizer that targets prostate-specific membrane antigen (BChl-PSMA) in combination with subtherapeutic near-infrared laser irradiation (750 nm) to enhance tumor nanoparticle delivery. The effects of BChl-PSMA-enabled PDT on tumor nanoparticle accumulation, therapeutic efficacy and intratumoral distribution were evaluated in a dual subcutaneous PSMA-positive prostate cancer mouse model. Accumulation of various types of nanoparticles was quantified by fluorescence spectrometry (Doxil®), gamma counting (^{64}Cu-labeled lipoprotein-like nanoparticles), and ICP-MS (gold).

Results and Discussion
We demonstrated that PDT pre-treatment with BChl-PSMA enhanced and accelerated accumulation of various organic (liposomes, lipoprotein-like) and inorganic (gold) nanoparticles in the laser treated tumor compared to the non-treated tumor in the same animal. Importantly, we established that a light dose of 50 J / cm^2 did not affect tumor vessel viability, which is essential for systemic drug delivery. Finally, we demonstrated the ability of targeted PDT pre-treatment to increase tumor accumulation of an FDA-approved nanomedicine (Doxil®) from 3.17 ± 0.59 to 7.19 ± 1.15 %ID / g, which translated into its improved therapeutic efficacy in a subcutaneous tumor model. Overall, the low molecular weight and long blood circulation time (~13 hours) of BChl-PSMA enables high tumor accumulation and homogeneous photosensitizer distribution. Therefore, this subtherapeutic targeted PDT improves access for larger drug-carrying nanomedicines to deeper layers of tumor tissue increasing the exposure of cancer cells to the drug.

Conclusions
In summary, targeted subtherapeutic PDT can provide a minimally-invasive strategy to enhance the tumor accumulation and therapeutic efficacy of FDA-approved nanoformulations.

> OC038. Oral Communication
Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

ROSE BENGAL-(KLAKLAK)2: A PHOTOSENSITISER-PEPTIDE CONJUGATE FOR TREATMENT OF MELANOMA

Authors: Simon Porter[U20], Simanpreet Dhillon[U20], John Callan[U20], Bridgeen Callan[U20]
Presenting Author: Simon Porter
1) Ulster University Coleraine

Introduction

Photodynamic therapy (PDT) has achieved notable success with marketed product Metvix(1) for treatment of superficial dermal carcinomas, however it is generally accepted that PDT has reduced efficacy against malignant melanoma . This is likely due to the presence of melanin, which competes with the photosensitiser for the absorption of photons. Responsible for the largest number of deaths from skin cancer(2), melanoma presents a significant treatment target; herein we present data demonstrating that a photosensitiser (Rose Bengal) with a peptide conjugate $(KLAKLAK)_2$ is effective against a mouse melanoma model.

Methods

The Rose Bengal-$(KLAKLAK)_2$ conjugate was synthesised in house using a solid-phase FMOC synthesis method and purified using reverse phase preparative HPLC. A mouse derived melanoma cell line (B16-F10-luc2) and MTT assay were used to investigate differences in efficacy of the peptide conjugate and the free Rose Bengal photosensitiser, using a 3-hour treatment time and white light for 1 minute (22.8 J/cm^2). Cell viability following treatments was also quantified using bioluminescence. To determine in vivo efficacy, B16 cells were implanted into SCID mice and allowed a 4-day growth period. Tumours were treated 1, 2, 7 and 9 days after the initial growth period with a 100 µL aliquot of 100 µM Rose Bengal or Rose Bengal-$(KLAKLAK)_2$ conjugate and white light for 3 minutes (68.4 J/cm^2) and tumour volumes monitored over a 12 day period.

Results

At several tested concentrations of free Rose Bengal there was no significant reduction in cell viability with 3 hours treatment time (Fig 1). Under the same conditions, the Rose Bengal-$(KLAKLAK)_2$ conjugate showed a significant reduction in viable cells of ~80 % when activated by white light, but with no toxicity in the light free controls (Fig 1). The in vivo results were similar, demonstrating that Rose Bengal alone had no significant reduction in tumour volume after 12 days (500 mm^3), while those treated with Rose Bengal-$(KLAKLAK)_2$ had a final tumour volume of <100 mm^3, highlighting the superior activity of the conjugate (Fig 2).

Conclusions

Conjugation of the Rose Bengal photosensitiser with the $(KLAKLAK)_2$ modality leads to a dramatic increase in efficacy against B16 melanoma cells both in vitro and in vivo. Current work is focused on the optimisation of drug quantity, dosing frequency and light activation time in vivo. Alternative routes of administration for RB-$(KLAKLAK)_2$ are also being investigated.

Acknowledgements

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> OC039. Oral Communication

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

PHOTODYNAMIC THERAPY (PDT) WITH TPCS2A/FIMAPORFIN IS EQUALLY EFFICIENT IN ^{ALDHDIM} AND ALDHbright COLON CANCER CELLS

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Aldehyde dehydrogenase (ALDH) enzymes are a group of enzymes with diverse activity, including vital role in detoxification of endogenous and exogenous aldehydes and is required for the conversion of retinol (vitamin A) to retinoic acid. Due to ALDH's high expression in normal stem cells where it is involved in differentiation and self-protection, ALDH is used as a marker for normal stem and progenitor cells. Interestingly, certain isoforms of ALDHs are also suggested to contribute in mediating cancer stem cells (CSC) capacities such as therapy resistance. The aim of this study was to evaluate the cytotoxic effect of $TPCS_{2a}$-PDT in cancer cells with high expression of ALDH ($ALDH^{bright}$ cells). $TPCS_{2a}$ (fimaporfin, PCI Biotech AS) is a clinical relevant photosensitizer used in photochemical internalization (PCI), which is a technology for intracellular drug delivery. In this study, both murine and human cancer cell lines were screened, by use of flow cytometry, for ALDH activity using the ALDEFLUOR assay. The human colon cancer cell line HT-29 was selected for further evaluation and sorted using the ALDEFLUOR assay in three populations: $ALDH^{bright}$, $ALDH^{dim}$ and bulk population. Sorted cells were then evaluated for response to $TPCS_{2a}$-PDT, chemo- and radiation therapy. Similar 5-FU and oxaliplatin sensitivity were found in $ALDH^{bright}$, $ALDH^{dim}$ and unsorted HT-29 cells. We show that $ALDH^{dim}$ cells are more sensitive to ionizing radiation compared to bulk and $ALDH^{bright}$ populations confirming existing reports. However, we found $TPCS_{2a}$-PDT to be equally efficient in both $ALDH^{bright}$ and $ALDH^{dim}$ populations. Our data indicate that $ALDH^{bright}$ CSC are sensitive to $TPCS_{2a}$-PDT as opposed to radiation therapy, and therefore further strengthen the use of $TPCS_{2a}$-based PCI of CSC-targeting therapeutics.

> OC040. Oral Communication

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

NOVEL THERANOSTIC PHOTOTHERAPEUTIC METALLOPORPHYRINS FOR PDT AND RADIOTHERAPY

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Aims

Approximately 50% of all cancer patients undergo radiotherapy as part of their treatment regime, the outcome of which can be devastating for patients with hypoxic solid tumours, which leads to poor prognosis.[1] While Zn(II) has been found to produce highly phototoxic porphyrins, electron-affinic Cu(II) porphyrins have been found to act as powerful radiosensitizers, demonstrating that porphyrins can be tuneable scaffolds. This represents an interesting photoactive multifunctional porphyrin capable of giving PDT activity as the free-base or Zn(II) chelate, as well as PDT and radiosensitising therapeutic properties when chelated to Cu(II). Additionally, the introduction of a PET isotope can confer the porphyrin with an imaging modality to give a multifunctional theranostic system

Results

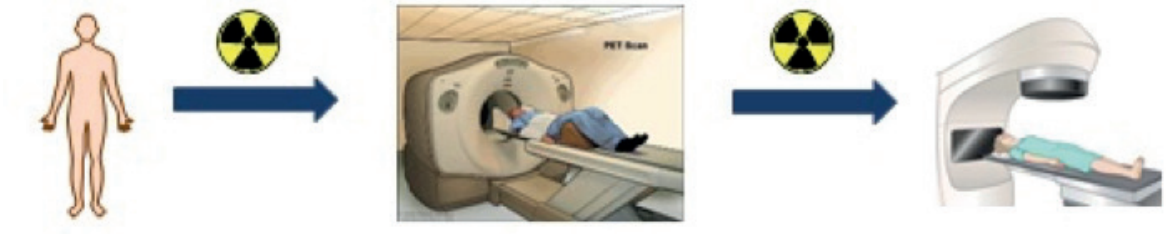
We have been investigating the synthesis of multifunctional theranostic phototherapeutic porphyrins which are capable of delivering a targeted therapeutic effect with potential for combined imaging and phototherapeutic modalities. We have synthesised an electron-affinic porphyrin which is capable of acting as a photosensitizer when chelated to Zn(II), but also as a radiosensitiser when chelated to Cu(II). We have been investigating the mechanism of action of these porphyrins as radiosensitizers through biological assays. Preliminary radiochemistry results demonstrate that the compound can be labelled with [^{18}F] and purified giving a theranostic agent.

Conclusion

We have successfully synthesised and biologically evaluated a multifunctional phototherapeutic porphyrin that is capable of acting as a photosensitizer or a radiosensitizer.

References

1. R. Baskar, K. A. Lee, R. Yeo and K. Yeoh, *Int. J. Med. Sci.*, 2012, **9**, 193–199.
2. J. A. O'Hara, E. B. Douple, M. J. Abrams, D. J. Picker, C. M. Giandomenico and J. F. Vollano, *Int. J. Radiat. Oncol.*, 1989, **16**, 1049–1052.



> OC041. Oral Communication
Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

PHOTODYNAMIC THERAPY AND PHOTOCHEMICAL INTERNALISATION TREATMENTS IN TUMOUROID MODELS OF OVARIAN CANCER

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Introduction
Compressed 3D collagen models have the potential to mimic the dense extracellular matrix (ECM) of a human tissue more closely than 2D cultures. These models allow the incorporation of stromal cells and matrix proteins and also take into account the interactions between the cancer cells and the matrix. We have developed a tumouroid 3D model that incorporates a central cancer mass surrounded by a stromal compartment, which enables visualisation of cancer cell invasion into the stroma.[1] Photochemical internalisation (PCI) uses sub-lethal photodynamic therapy (PDT) to enhance the delivery of therapeutic agents that are prone to endolysosomal degradation to their intracellular target sites of action. The release of the endocystosed compound occurs when the vesicle membrane ruptures due to photoactivation of a photosensitiser by light and generation of reactive oxygen species.

Aim
To investigate the efficacy of PDT and PCI on compressed 3D collagen tumouroid models of ovarian cancer.

Materials and Methods
Tumouroid models of ovarian cancer were created using HEY (ovarian cancer cells), HDFs (human dermal fibroblasts) and HUVECs (human umbilical vein endothelial cells). The tumouroid model comprises a central zone c. 4 mm diameter of cancer cells embedded in collagen surrounded by a stromal compartment containing the HDFs and HUVECs in collagen. The tumouroids were treated either the photosensitiser (disulfonated tetraphenyl porphine) alone or a macromolecular toxin (saporin) alone or a combination of both drugs and were incubated for 24 hours, and then exposed to light. Images were obtained using fluorescence microscopy with vital stains to observe the changes in the tumouroid post-treatment.

Results and Discussion
Treatment using PDT only caused a reduction in cancer cells invading the stroma and HDFs in comparison to the control and saporin only treated constructs. More extensive destruction of the cancer cells in the central cancer mass and of those invading the stroma was observed in PCI treated constructs along with destruction of HUVEC and HDF cells in the stroma. Thus, PCI exerts greater inhibition on cancer cell invasion into the stroma than PDT but also causes damage to the stromal cells. These results demonstrate the differential response between PDT and PCI and their effect on invasive cancers, and highlight the utility of this 3D cancer model for testing PDT and PCI.

Reference

1. Magdeldin T, Lopez- Davila V, Pape J, Cameron G.W.W, Emberton M, Loizidou M, Cheema U. (2017),Engineering a vascularised 3D *in vitro* model of cancer progression, *Scientific Reports*, 7:44045 | DOI: 10.1038/srep44045.

> OC042. Oral Communication
Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

INDOCYANINE GREEN LOADED APTMS COATED SPIONs FOR DUAL PHOTOTHERAPY OF CANCER AND BIOFILMS

Authors: Kubra Bilici[Koc U], Abdullah Muti[Koc U], Nazli Ataç[Koc U], Alphan Sennaroglu[Koc U], Füsun Can[Koc U], Havva Yagci Acar[KocU]
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Introduction
Superparamagnetic iron oxide nanoparticles (SPIONs) have been used in magnetic hyperthermia and MRI for many years and recently, its PTT potential has been discovered [1, 2]. Indocyanine green (ICG) is an FDA approved dye and recognized as a sensitizer in PDT in recent years. In this work, ICG@APTMS SPIONs were used for combined PTT and PDT via a single laser treatment. Detailed analysis of PTT potential of APTMS-SPIONs, effect of PTT/PDT combination on tumor cells as well as on biofilms will be discussed.

Methods
APTMS@SPIONs were synthesized by co-precipitation method using iron salts. Then, ICG was electrostatically loaded to APTMS@SPIONs. The PTT potential of NPs were investigated as a function of NP concentration and laser power using 785 nm diod laser. In vitro studies were performed on MCF7 and HT29 without and with laser treatment. The antimicrobial activity was studied on planktonic and sessile cells of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus epidermidis*. The biofilms were formed on polystyrene 96-well plates with Tryptic Soy Broth at 24 hours. The number of viable cells was determined by colony count assay on tryptic soy agar.

Results & discussions
A dose and laser intensity dependent temperature increase was observed in APTMS@SPIONs. ICG@APTMS SPIONs showed better stability and temperature increase than free ICG. Fluorescence images of free ICG and ICG@APTMS SPIONs showed high internalization on both HT29 and MCF7 and almost complete cell death after laser treatment of NP treated cells. Cell death was confirmed with MTT assay and Live/dead kit. Combination therapy was confirmed by studying the ROS level of cells treated with ICG loaded and ICG free NPs after laser treatment. On planktonic cells of all bacteria, ICG at doses of 10 µg/ml and 25 µg/ml caused 5-log decrease in number of viable cells and total inhibition of growth was observed with 5, 10 and 25 µg/ml ICG@APTMS SPIONs after application of PTT. There was no growth inhibition in cells which were not exposed to PTT. On sessile cells in biofilm, both ICG and ICG@APTMS SPIONs caused 4-log decrease in growth at doses of 10 and 25 µg/ml when stimulated with PTT. No growth inhibition was found without PTT

Conclusions
Small, cationic APTMS@SPIONs are shown as good candidates for local temperature increase upon irradiation at NIR wavelength. ICG loading provided optical imaging and PDT potential to these SPIONs. Hence, PDT/PTT combination and excessive antitumor and antibacterial activity with a single laser treatment was achieved both in cancer cells and biofilms

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> P043. Poster

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

ROS GENERABLE MICELLES THAT ESCAPABLE ENDOSOME AND LYSOSOME FOR TREAT CANCER

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Micelle is one of the drug delivery system for treat intractable diseases, such as cancer. Also, micelles regulated drug release via various external stimuli such as pH, temperature, light, etc. Reactive oxygen species (ROS) is one of the stimuli for disrupt of micelles [1]. Photosensitizer (PS) can be used as ROS generable materials. Endosome and lysosome are barriers to completing enough therapeutic efficacy. So, endosome/lysosome escape are necessary to delivery drug into cells. To overcome this barrier, Endosome and lysosome was disrupted by photochemical internalization. Its mechanism is based on the disruption of cellular membrane by ROS such as singlet oxygen ($^1O_2$). [1]

We developed ROS generable micelles (RGMs) that control drug release and escape endosome and lysosome via light expose. RGMs were composed PS and ROS sensitive polymer. We confirm ROS producing ability of RGMs by using singlet oxygen sensor green (SOSG). Also, we confirm drug release of RGMs when laser irradiation. In vitro test, we confirm accumulation of RGMs into endosome and lysosome. And confirm escape endosome and lysosome of RGMs when laser irradiation. We confirm cytotoxicity of RGMs with laser irradiation and without laser irradiation. In vivo test, we confirm change of tumor volume when treat RGMs with laser irradiation. Also, we confirm change of body weight of tumor bearing mice when treat RGMs with irradiation. And, we confirm histological change of cancer via H&E and TUNEL assay. We develop ROS generable micelles that escapable endosome and lysosome for cancer treatment. RGMs can accumulate in cancer cells. Also, RGMs can escape endosome and lysosome via laser irradiation. RGMs has cancer treatment efficacy via in vivo test.

References

[1] H. Otsuka, Y. Nagasaki and K. Kataoka, Adv. Drug Delivery Rev., 2012, 64, 246.
[2] H. Chen, L. Xiao, Y. Anraku, P. Mi, X. Liu, H. Cabral, A. Inoue, T. Nomoto, A. Kishimura and N. Nishiyama, J. Am. Chem. Soc., 2014, 136, 157.
[3] H. C. Yen, H. Cabral, P. Mi, K. Toh, Y. Matsumoto, X. Liu, H. Koori, A. Kim, K. Miyazaki, Y. Miura, N. Nishiyama and K. Kataoka, ACS Nano, 2014, 8, 11591.

> P044. Poster

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

ENHANCED CANCER TREATMENT USING BIMODAL NANOPARTICLES WITH PHOTOSENSITIZER-CONJUGATE POLYSACCHARIDE SHELL AND NATURE-DERIVED PHOTOTHERMAL POLYMER CORE

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Cancer treatment has been studied variously, among which photodynamic therapy (PDT) is a promising method because photosensitizer (PS) has powerful reactive oxygen species (ROS) generation capacity that can kill cancer cells very effectively [1]. However, the conventional PDT has some limitations because PS is barely soluble in aqueous phase, do not have selectivity between lesion and normal tissues, may cause unexpected ROS generation by light condition [2]. To overcome the limitations mentioned above, we designed targetable nanoparticles consist of photosensitizer-polysaccharide shell and photothermal polymer core (PSPC), synthesized by the chemical oxidation. The polysaccharide can recognize a specific receptor of cancer cell surface. Conjugated PSs with polysaccharide in PSPC are quenched together so they cannot produce ROS before degradation by enzyme in cancer cells. The core polymer is nature-derived and has photothermal effect under laser irradiation, which is expected synergetic effect with PS.

Our PSPC is uniform spherical, has approximately 120 nm size, evenly dispersed in aqueous phase confirmed by DLS and SEM images. The photoactivity of PS in PSPC is maintained and enhanced in cell medium because of enhanced hydrophilicity. Also, PSPC can target cancer cells and selectively internalization into the cells evaluated by FACS and CLSM. Anticancer activity of PSPC is conducted, showed an excellent therapeutic effect in our *in vivo* system. These results suggest our PSPC can be targetable, controllable combination photodynamic therapy agent for enhanced cancer treatment.

References

[1] Agostinis, P.; Berg, K.; Cengel, K. A.; Foster, T. H.; Girotti, A. W.; Gollnick, S. O.; Hahn, S. M.; Hamblin, M. R.; Juzeniene, A.; Kessel, D.; Korbelik, M.; Moan, J.; Mroz, P.; Nowis, D.; Piette, J.; Wilson, B. C.; Golab, J. Photodynamic Therapy of Cancer: an Update. Ca-Cancer J. Clin. 2011, 61, 250−281.
[2] Park, W.; Park, S. J.; Na, K. The Controlled Photoactivity of Nanoparticles Derived from Ionic Interactions between a Water Soluble Polymeric Photosensitizer and Polysaccharide Quencher. Biomaterials 2011, 32, 8261−8270.

> P045. Poster

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

SIMULTANEOUS THERAPY USING TEMPERATURE AND LIGHT BY THERMO-SENSITIVE BIOPOLYMERIC PHOTOSENSITIZER FOR EFFICIENT CANCER THERAPY

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Photodynamic therapy (PDT) based on light and photosensitizers (PSs) has been used for various diseases effective treatment and attracted attention as a promising treatment for cancer therapy. However, the clinical application of PDT has been limited because of its drawbacks caused by poor cancer accumulation and unregulated toxicity of PSs being used in PDT. To resolve these drawbacks, we have we have developed thermo-sensitive biopolymeric photosensitizer (TSBPS) that photoactivity able to be regulated by external temperature.

TSBPS was synthesized by conjugation of PS with thermo-sensitive polymer, hydroxypropyl cellulose (HPC) which is biocompatible cellulose. Conformation changes of TSBPS were confirmed in UV–vis spectrophotometer analysis and synchrotron small-angle X-ray scattering (SAXS) at various temperatures. In addition, therapeutic efficiency of TSBPS through dual therapy of PDT and thermal therapy was analyzed against pancreatic cancer cell.

The intermolecular interaction changes of PS molecules in TSBPS at different temperature was investigated through SAXS. TSBPS has a lower critical solution temperature (LCST) in water (43.0 ± 1.0 ℃) and occurs a phase change from random-coil conformation (hydrophilic) to collapsed form (hydrophobic) as temperature increases above the LCST. As a result, PS molecules connected quite close by π–π stacking was quenched in TSBPS under LCST. Biopolymer was easily transited to an active monomeric state by the thermal-induced phase transition above LCST. In this reason, singlet oxygen generation and fluorescent emission of TSBPS were significantly increased at hyperthermia condition than at physiological condition. Also, In vitro cytotoxicity data proved the additional cancer treatment effect at 45 °C with laser irradiation.

We have developed thermo-sensitive biopolymeric photosensitizer (TSBPS) to regulate photoactivity of PS and advanced cancer therapeutic effect by simultaneous photodynamic and thermal therapy. TSBPS shows notable advanced singlet oxygen generation and fluorescent emission at hyperthermia condition than at physiological condition. Due to this results, in vitro cytotoxicity data showed that TSBPS greatly increased the cancer cell killing effect at hyperthermia condition with laser irradiation.

References

1. Henderson, B. W.; Dougherty, T. J. Photochem. Photobiol. 1992, 55, 145.
2. Lovell, J. F.; Liu, T. W.; Chen, J.; Zheng, G. Chem. Rev. 2010, 110, 2839.
3. Koide, Y.; Urano, Y.; Yatsushige, A.; Hanaoka, K.; Terai, T.; Nagano, T. J. Am. Chem. Soc. 2009, 131, 6058.

> P046. Poster

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

CANCER THERAPY USING MULTIFUNCTIONAL MAGNETIC NANOPARTICLE

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Magnetic nanoparticles are used in a variety of fields such as magnetic resonance imaging (MRI) materials and cancer treatment. However, magnetic nanoparticles have some difficulties to achieve better therapeutic effects. One of the limitations is tumor-specific accumulation. The important condition for becoming an effective anticancer drug is the accumulation of cancer sites. But, most of the agents are not specific to cancer. This limitation causes repeated injection of drugs and increase in side effects.

We designed magnetic nanoparticles composed of a polymer coupled with a photosensitizer (MPPS). MPPS was synthesized by hydrolysis using an amine. We confirmed that MPPS was synthesized as ^{1}H – NMR, size and zeta potential. Polymer have cancer-specific residues. MPPS can target cancer via cancer-specific residues mediated endocytosis and therapeutic effect increase. And photosensitizer enables photodynamic therapy (PDT) that induces the death of cancer cells by generating reactive oxygen during laser irradiation. In addition, MPPS has a temperature rise of up to 43 ° C depending on the magnetic field and is highly soluble in water. ROS and high temperature make PDT and hyperthermal therapy possible. The advantage of PDT and hyperthermal therapy is that it is a non-invasive treatment, which increases the quality of life for the patient. Therefore, dual therapy can provide effective cancer treatment.

We magnificently detected cancers in mice through MRI and optical imaging. This tested in tumor bearing mouse models. The inhibitory effect of MPPS on tumor growth was monitored for each of PDT only, hyperthermal therapy only and combination therapy. Single therapies a little inhibited cancer growth. But dual therapy noticeably suppressed tumor growth through synergies that could result in vessel damage.

As a result, we proved MPPS, a multifunctional combination chemotherapeutic agent containing magnetic nanoparticles with higher biocompatibility and magnetic properties. MRI and fluorescence imaging due to MPPS enable the double diagnosis of tumors. In addition, the dual treatment of PDT and hyperthermal therapy improved tumor growth inhibition efficacy. Consequently, the PDT and hyperthermal therapy complex tumor therapy strategy based on MPPS can develop cancer nanotechnology confidently and be very useful for clinical applications

References

[1] R. Hachani, M. Lowdell, M. Birchall and N. T. K. Thanh, *Nanoscale*, 2013, 5, 11362 -11373
[2] H. Xu , Z. P. Aguilar , L. Yang , M. Kuang , H. Duan , Y. Xiong , H. Wei and A. Wang , *Biomaterials*, 2011, **32** , 9758 -9765
[3] A. Tomitaka, A. Hirukawa, T. Yamada, S. Morishita and Y. Takemura, J. Magn. Magn. *Mater.*, 2009, 321, 1482 -1484

> P047. Poster

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

PHOTODYNAMIC AND ANTIBIOTIC TREATMENT OF ACNE WITH LIPASE-RESPONSIVE LIPOSOME WITH ENHANCED ANTIBACTERIAL PERFORMANCE

Authors: Songhee Jeong[1], Jonghwan Lee[1], Byeong Nam Im[1], Kun Na[1*]
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Acne vulgaris is a normal skin disease that occurs when the hair follicles are blocked by oil and dead corneous cells from the skin. And *Propionibacterium acnes* (*P. acnes*) is significantly associated with acne development. Although conventional acne treatments are being treated with antibiotics, there is another issue of antibiotic resistant *P. acnes* arising from the use of overdose antibiotics [1].

In this study, an alternative way is developed to treat acne using lipase-reactive liposomes with photosensitizers and antibiotics (LRLPA). The LRLPA is coated by maltotriose units based polysaccharide polymer-photosensitizer(MPP-PS) conjugates on the antibiotics loaded liposome. The physical properties of the LRLPA were confirmed by the size and transmission electron microscope (TEM) image of the liposome. The ester bond of MPP-PSs, which constitutes the liposome, is cleaved by the lipase secreted by *P. acnes.* And it is possible to disrupt LRLPA structure and release quenched PS and antibiotics from the LRLPA. And recovered photoactivity of PS was confirmed by fluorescence intensity according to lipase concentration. And the antibacterial effect of antibiotics and photodynamic therapeutic effect of reactive oxygen species(ROS) from the PS in laser irradiation was confirmed by colony forming units(CFU) assay in vitro. Also the antibacterial effect of LRLPA was demonstrated by observing changes in size of acne in a mouse model and significantly reducing the number of colony of acne after treatment. As a result, *P. acnes* selective and excellent antimicrobial effect of LRLPA confirmed the possibility of an acne remedy having potential to be an alternative to conventional antibiotic treatment.[2]

References

[1] Jeong, Songhee, et al. “Combined photodynamic and antibiotic therapy for skin disorder via lipase-sensitive liposomes with enhanced antimicrobial performance.” *Biomaterials* 141 (2017): 243-250.
[2] Park, Hyung, et al. “Lipase-Sensitive Transfersomes Based on Photosensitizer/Polymerizable Lipid Conjugate for Selective Antimicrobial Photodynamic Therapy of Acne.” *Advanced healthcare materials* 5.24 (2016): 3139-3147.

> P048. Poster

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

PRESENCE OF ENDOGENOUS PORPHYRINS AND ANTIMICROBIAL BLUE LIGHT RENDERS ACINETOBACTER BAUMANNII CLINICAL ISOLATES SUSCEPTIBLE TO ANTIMICROBIALS

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Antimicrobial resistance (AMR) crisis has forced the intensive findings into alternative approaches and isolation of new sources of secondary metabolites with biocidal activity. The photobiological sciences also are determined into finding the solutions of still increasing AMR, thus the antimicrobial blue light inactivation (aBL) can be used successfully in eradication of multidrug resistant Gram positive as well as Gram negative microorganisms. The presence of endogenous porphyrins in bacterial cells were described in literature data as a major factor which is highly responsible for eradication of pathogens e.g. *S. aureus, E. coli, P. aeruginosa* after exposure to visible blue light.

The endogenous porphyrins were extracted from two extensively drug resistant, clinical isolates of *A. baumannii* and its presence was examined with UV-VIS spectroscopy and mass spectrometry (MALDI TOF MS). Furthermore, the experiments involving the aBL were performed and the determination of the influence of the blue light and identified porphyrins were performed with recommended methods for synergy testing (e.g. E-test, checkerboard assay, post antibiotic effect). The investigation of the production of hydroxyl radicals and singlet oxygen in the presence of light and antibiotics were performed with aPF (3'-(p-aminophenyl) fluorescein) and SOGS (Singlet Oxygen Sensor Green) indicators.

Spectroscopic and spectrometric method evidenced the presence of endogenous porphyrins in *Acinetobacter* spp. cells. Moreover, application of sub-lethal doses of aBL and involvement of porphyrins resulted in re-sensitization of the tested strains to antimicrobials (e.g. doxycycline, imipenem, colistin) which was evidenced with multiple methods. Furthermore, the aBL in presence of colistin and doxycycline resulted in the increased production of reactive oxygen species and singlet oxygen.

Overall the conducted experiments confirmed the influence of the presence of endogenous porphyrins in *Acinetobacter baumannnii* and evidenced the effectiveness of application of aBL in eradication and re-sensitization resistant strains to routinely used antimicrobial agents.

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> P049. Poster

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

METABOLIC CHARACTERIZATION OF PDT RESISTANT BASAL CELL CARCINOMA AND METFORMIN LIKE ADJUVANT

Authors: Marta Mascaraque[1], Pablo Delgado-Wicke[1], Tamara Gracia-Cazaña[2], Yolanda Gilaberte[3], Ángeles Juarranz[1]
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Photodynamic therapy (PDT) is used for the treatment of several types of Non-Melanoma Skin Cancer (NMSC) although sometimes resistant cells, responsible for relapses, may appear after treatment. Normal differentiated cells depend primarily on mitochondrial oxidative phosphorylation to generate energy, but cancer cells change this metabolism to an aerobic glycolysis (Warburg effect), which could influence in the response to antitumor drugs. Here, we have evaluated the potential metabolism changes that occur in resistant to PDT of basal carcinoma cells (BCC). The mouse cell lines ASZ and CSZ were used, both heterozygous for *ptch1*. The cells, called parental (P), were subjected to 10 PDT cycles (1 mM methyl-aminolevulinate, followed by red light irradiation) to obtain resistant cells (10G). Resistant cells were inoculated in immunosuppressed mice, the induced tumors were sub-cultured by explants and a cell population called 10GT was obtained. We first confirmed the resistance of the different cells to PDT. In addition, we have analysed by western blot and immunofluorescence, the expression of different metabolic markers (bioenergetic signature (β-F1-ATPase/GAPDH) and PKM2) in the cell populations. The results showed that the expression of these factors was lower in PDT resistant than in parental cells. Therefore, we combined PDT with metformin, an antidiabetic type II compound. The results obtained showed a significant increase in cell death after the combined treatment comparing to that induced by PDT or metformin alone. In addition, we have evaluated the changes induced in elements of mTOR pathway after treatments since it is known the metformin increase the expression of AMPK, an inhibitor of mTOR. Taken together the obtained results, we propose metformin as an excellent coadjuvant treatment of PDT.

> P050. Poster

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

COMBINING VERTEPORFIN-PDT AND 5-AZA-2′-DEOXYCYTIDINE FOR NEO-ADJUVANT TREATMENT OF BREAST CANCER

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Introduction

Primary breast cancer treatment relying on surgery with the use of neo-adjuvant therapies has been a long-established approach. However, side-effects and varying efficacy have led to the search for novel therapies with better outcomes. Combinatory strategies of various therapeutic modalities have shown promise improving treatment outcomes. In this project, we have investigated the potential of photodynamic therapy (PDT) as a novel neo-adjuvant treatment alone and in combination with the cytotoxic agent 5-Aza-2\'-deoxycytidine (5-ADC) against breast cancer cells.

Methods

The murine mammary carcinoma cell line 4T1 was used for the experimental *in vitro* study as it very closely mimic stage IV human breast cancer. We determined the optimum dose of liposomal verteporfin-PDT monotherapy required to inactivate cancer cells by several proliferation/viability assays. We compared the cytotoxic effect and morphological changes induced by PDT alone and in combination with the chemotherapeutic and immunomodulatory agent 5-ADC by qPCR, western blot and fluorescence microscopy. The enhanced antitumour response induced by the combination of verteporfin-PDT with 5-ADC was validated using an orthotopic 4T1 breast cancer mouse model. Immune activation after treatment was investigated by flow cytometry and validated by immunohistochemistry.

Results and Discussion

Verteporfin-PDT *in vitro* treatment resulted in rapid induction of cell death while 5-ADC treatment elicited delayed cytotoxic effects. Combination treatment induced synergistic tumour suppression compared to monotherapies. *In vivo* local and distant effects of liposomal verteporfin-PDT treatment were demonstrated in comparison to 5ADC alone. Enhanced antitumour effects were also observed using the combination strategy. Well-demarcated PDT tumour damage with clear necrotic margins on histopathology assessment occurred in PDT monotherapy and in the combination treatment group. Flow cytometry and gene expression analysis performed in samples obtained from PDT-treated mice provided evidence for s PDT-mediated activation of innate immunity and absence of metastases.

Conclusions

The results suggest that the use of PDT as adjuvant therapy in combination with other cytotoxic agents may be more effective in the treatment of primary breast cancer and further pre-clinical and clinical investigations are needed to further elucidate its benefits.

Acknowledgements

The financial support from the Royal Free Charity, the Peter Samuel Royal Free Fund and the Ramón Areces Foundation is gratefully acknowledged.

Conflicts of interest

The authors declare no competing financial interest.

> P051. Poster

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

SYNERGISTIC AND NON-TOXIC EFFECT OF PDT AND DOXYCYCLINE COMBINATION AGAINST HELICOBACTER PYLORI

Authors: Paola Faraoni[1], Alessio Gnerucci[1], Ilaria Baccani[2], Alberto Antonelli[2], Matilde Marini[2], Barbara Orsini[1], Patrizia Pecile[3], Gian Maria Rossolini[2,3], Franco Fusi[1], Giovanni Romano[1]
Presenting Author: Giovanni Romano
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Introduction

In recent years, the emergence of an increasing number of multidrug resistant *Helicobacter pylori* -associated infections leads to the urgent search for novel therapeutic solutions [1, 2]. In this regard, different PDT treatment strategies have been proposed, all characterized by the absence of external photosensitizers, due to endogenous production of photoactive porphyrins by *H. pylori* itself, notably protoporphyrin IX (PPIX).

Methods

In this work the possible synergy between doxycycline and therapeutic light was investigated in three different *Helicobacter pylori* (*H. pylori*) strains (ATCC 700392, ATCC 43504 and ATCC 49503) susceptible to this antibiotic. Moreover, to evaluate the possible side effects of this therapeutic treatment, the cytotoxicity of this combination with and without PPIX on AGS cells (ATCC CRL-1739), was evaluated [3]. Bacterial cultures were grown on solid medium either containing or not doxycycline at sub-inhibitory concentrations, and irradiated for 10, 20, 30 minutes with a 400nm-peaked light source (4.8 mW/cm^2). Viability was evaluated by post-treatment CFU counting. The phototoxicity tests on AGS cells were performed incubating with or without doxycycline for 72 hours at the above-mentioned concentrations and subsequently overnight with or without 50 nM of PPIX, a concentration higher than the estimated amount of PPIX released *in vitro* by *H. pylori* in culture medium (12-42 nM, literature data). Irradiation was performed with the same parameters used with *H. pylori* cultures and post-treatment cell viability was evaluated by MTT assay. Controls corresponding to irradiated cell samples only were prepared for comparison.

Results and Discussion

Indications of an antibacterial synergistic effect were obtained when both antibiotic and light treatments were performed, showing an enhancement of the photokilling efficacy. No significant toxic effects in AGS cells were observed using PDT, doxycycline and PPIX alone and in combination between them under the same conditions of exposure.

Conclusions

The combination of doxycycline and PDT against *H. pylori* strains could be considered as an interesting therapeutic option associated with no toxicity for the healthy gastric mucosa.

Acknowledgements

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All authors declare to have no conflict of interest.

References

[1] W.D. Chey et al. (2018) *Am J Gastroenterol* 113(7): 1102
[2] G. Tortora et al., (2016) *IEEE/ASME Transactions on Mechatronics*, 21(4): 1935-1942.
[3] P. Faraoni et al., (2018) *J. Photochem. Photobiol, B: Biol* 186: 107-115.

> P052. Poster

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

SYNTHESIS OF BREAKABLE SINGLET OXYGEN (1O2) MESOPOROUS SILICA NANOPARTICLES (MSNP's)

Authors: Jaume Nos[1], Santi Nonell[1], Lucrezia Preto[2]
Presenting Author: Jaume Nos
1) IQS School of Engineering Universitat Ramon Llull 2) Università di Parma

A novel bimodal mesoporous silica nanoparticle (MSNP) has been developed to deliver the adsorbed photosensitizer (PS) and oncological drug, namely Methylene blue (MB) and Doxorrubicin (DOX). The MSNP's were synthesized *via* a modified base-catalyzed Stöber process involving the mixture and copolymerization of two silanes: tetraethyl orthosilicate (TEOS) and a breakable singlet oxygen ($^1O_2$) silane (molar ratio of 70:30 in Si source[1]).

DOX delivery has been assessed in the presence and absence of MB showing that there is no DOX delivery when the PS is not present in the nanoparticle. $^1O_2$, formed when the PS was irradiated at different times by a red LED at 661 nm, triggers the delivery of DOX because of the scissile $^1O_2$ silane moiety.

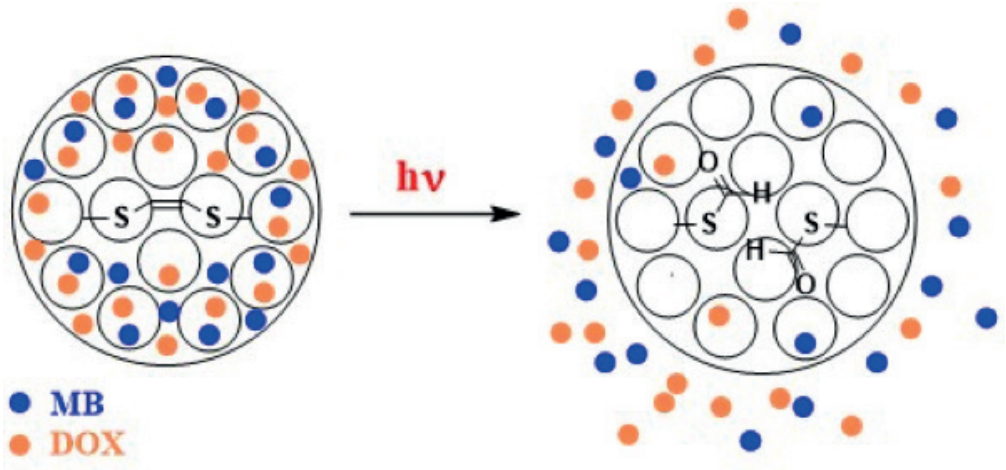
Irradiation of the nano-delivery system with red light leads to the controlled release of DOX from the MSNP.

Acknowlegments

This work has been supported by grant CTQ2016-78454-C2-1-R from the Spanish Ministerio de Economía y Competitividad.

References

[1] Laura Maggini, E. et al., Breakable Mesoporous Silica Nanoparticles for Targeted Drug Delivery, *Nanoscale* **2016**, 8 (13), 7240-7247



> P053. Poster

Symposium PDT-3 Combination of PDT with other treatment modalities (Elena Reddi)

SINGLET OXYGEN CONTROLLED DRUG RELEASE OF BIMODAL DOXORUBICIN-METHYLENE BLUE-MSNPs

Authors: Esther Lleixà[IQS], Elena de las Heras[IQS], Santi Nonell[IQS], Montserrat Agut[IQS]
Presenting Author:Esther Lleixà
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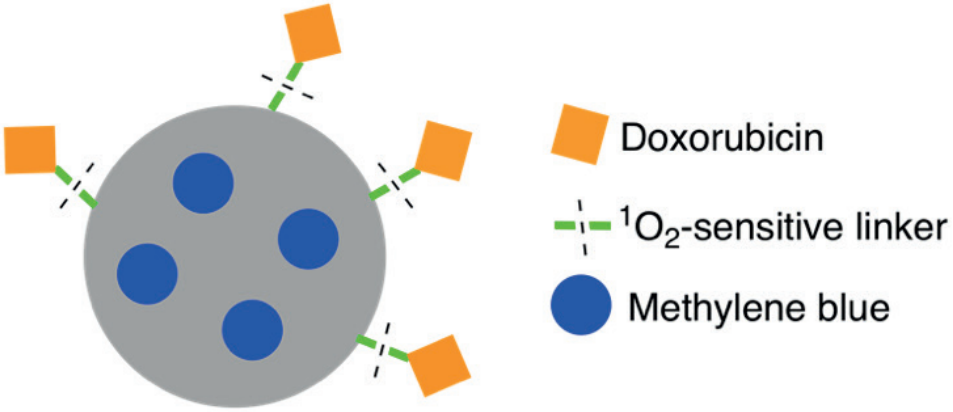
Drug-nano-delivery systems have enhanced the safety of highly cytotoxic treatments. However, the covalent bonds between the drug and the nanocarrier precludes its optimum subcellular localization and therefore limiting the effect of the therapy. To overcome this limitation, the aim of the present research is to introduce a cleavable linker by light mediated singlet oxygen ($^1O_2$) generation in order to allow the release of a covalently attached chemotherapeutic agent. To achieve this goal, the oncologic compound doxorubicin (Dox) has been attached to an asymmetric $^1O_2$-sensitive linker in three steps with a 2% overall yield. Thiolized mesoporous silica nanoparticles (MSNPs) have been functionalized with the synthesized Dox-cleavable linker. The photosensitizer methylene blue has been adsorbed to the resulting MSNPs in order to generate the $^1O_2$ capable to release the Dox. All the prepared MSNPs have been characterized physicochemically. Furthermore, the novel bimodal MSNPs successfully release the Dox upon irradiation with red light. Consequently, the attachment of a chemotherapeutic agent by means of a $^1O_2$ cleavable linker could control the release of the cytotoxic drug from the nano-delivery system, which has a tremendous potential in Photodynamic Therapy.



> IL102. Invited Lecture
Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

ANTIMICROBIAL PHOTODYNAMIC THERAPY FOR INACTIVATION OF BACTERIAL BIOFILMS

Authors: Fabian Cieplik[1], Karl-Anton Hiller[1], Tim Maisch[2]
Presenting Author: Fabian Cieplik
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Prevention and control of biofilm-growing microorganisms are a serious challenge for public health as biofilms are causative for more than 80% of all human infections. With respect to increasing numbers of drug-resistant pathogens all over the world there is a pressing need for development of strategies that are capable of inactivating bacterial biofilms with less risk of developing resistances in pathogens. In light of this, a promising alternative could be the antimicrobial photodynamic therapy (aPDT). The lethal effect of aPDT is based on the principle that visible light activates a photosensitizer (PS), leading to the formation of reactive oxygen species, e.g. singlet oxygen or oxygen radicals, that kill bacteria immediately during illumination by an oxidative burst.

While there are many studies that have shown the high antimicrobial potential of aPDT towards planktonic bacteria, it is by far more complicated when it comes to biofilms, where conflicting results have been reported.

Theoretically, for reaching a high efficacy towards biofilms, an aPDT system must combine two key features: 1) a PS must be designed in a way that allows its penetration throughout the biofilm and its matrix without being inactivated and 2) light must reach these PS molecules in the all layers of biofilms.

The aim of this talk is to summarize results from recent *in vitro*studies as well as clinical trials, to discuss potential limitations of aPDT when it comes to inactivation of biofilms and to give an overview about potential fields for clinical application of aPDT.

> IL103. Invited Lecture
Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

MUST HAVE OR NICE TO HAVE- MATHEMATICAL ASPECTS FOR ANALYSIS OF ANTIMICROBIAL PHOTODYNAMIC THERAPY DATA

Authors: Karl-Anton Hiller[1], Fabian Cieplik[1], Tim Maisch[2]
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Interinstitutional cooperation of a medical or biological research worker with less particular mathematical expertise but with the ability to follow straight-forward formulae on the one side and a professional mathematician or statistician on the other side needs to use a common language. Core issues are the knowledge of simple to highly sophisticated terms from each of both disciplines. A great challenge is the statistical treatment of data collected from studies dealing with the influence of certain substances on the state of being of bacterial cells, e.g. the photodynamic inactivation of bacteria (aPDT) grown in multi-species biofilms.

Aim of the talk is to remember basic statistical ideas for providing the audience with tools to analyze and simply present results of their sophisticated inactivation data.

Statistical basics will be provided, followed by general considerations to present aPDT results. Finally a strategy to statistically evaluate experiments scanning the interaction of two substances at various concentrations on bacteria (e.g.: checkerboard experiments) will be presented. Within this strategy, the definition of an appropriate end point (“optimal effective concentration combination”; OECC) is presented. Based on one and two dimensional fits an OECC will be derived from experimental data.

> IL104. Invited Lecture
Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

HUMAN TOPICAL ANTIMICROBIAL PHOTODYNAMIC THERAPY (aPDT)

Authors: Alison Mackay[1], Mark Farrar[2], Lesley Rhodes[2,1]
Presenting Author: Alison Mackay
1) Salford Royal NHS Foundation Trust 2) University of Manchester

A rising prevalence of antimicrobial resistance led the WHO to launch a global action plan to address this significant threat to human health. The plan prioritises vaccination against bacteria and viruses, and the collation of information using a global database, while emphasising the optimal use of antimicrobials. There have been limited localized geographical improvements with certain pathogens. However, improved strategies are required for widespread progress, with a particular potential role for antimicrobial photodynamic therapy (aPDT) as this multi-targeted therapy does not cause microbial resistance, nor is it affected by a drug resistant status.

Use of acridine and daylight to kill protozoa was described in 1900, with the first dermatological application in 1905 comprising topical eosin and daylight to treat mycobacterial skin infection (lupus vulgaris). More recently, many experimental studies have been performed to examine the potential for use of aPDT in infections caused by microorganisms including gram-positive and gram-negative bacteria, fungi and viruses. Use of a wide range of groups of photosensitisers in aPDT, with action spectra facilitating deep effect is being explored, and potential synergistic action by adjunctive agents. Mechanisms of action of aPDT include not only direct microbial inactivation, but also stimulation of host immune responses.

As yet, relatively little of this scientific progress has translated into clinical studies in human skin. However, promise has been seen in a range of areas, including in leg ulcers and diabetic ulcers, where reducing the commensal bacterial load has also been associated with ulcer healing. aPDT has been applied in localized cutaneous viral infections due to herpes and papilloma viruses, and also in acne, where it is unclear how much of the therapeutic effect can be attributed to the antimicrobial action. Success has been seen in anti-fungal applications with candida and dermatophyte infections including onychomycosis. aPDT has now reached the stage of being highlighted in guidelines for application in the form of ALA/MAL-PDT in acne, warts and cutaneous leishmaniasis. These may confer the benefit of a wider therapeutic window, i.e. greater safety compared with standard therapies.

> IL105. Invited Lecture
Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

ANTIFUNGAL PHOTODYNAMIC TREATMENT: FROM CANDIDA AND TRICHOPHYTON SPP. TO NEW EMERGING PATHOGENS

Authors: Gilberto Ú.L. Braga[1], Mark Wainwright[2]
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2) School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK

Fungal diseases became a major medical problem in the second half of the 20th century. The fungal kingdom is a numerous and diverse group. *Candida*, *Trichophyton* and *Aspergillus* spp. are well known human pathogens, but others belonging to other genera have emerged as important pathogens with increased virulence and resistance to antifungal agents. Fungal pathogenic species are able to form different cell types and specialized structures during infection such as yeasts, hyphae and spores. Most species are also able to form biofilms, which are usually more resistant to antifungal agents. Fungi can cause localized infections in different organs and structures, such as skin, nails, hair, lungs and the central nervous system, as well as disseminated infections. Therefore, Antifungal Photodynamic Treatment (APDT) deals with a myriad of situations that should be studied on a case by case basis. Novel photosensitizers (PS) have been synthesized and used *in vitro* against several species of “old” and “new” pathogenic fungi. PS more effective for each species and fungal structure have been identified. However, most APDT studies focus on a single photosensitizer class and only few compare the effectiveness of different classes. Phenothiazinium derivatives, porphyrins and phthalocyanines are among the most studied PS. Mechanistic studies have demonstrated the interaction of PS with different cell types and fungal structures, as well as the effects of the APDT on subcellular organelles and structures, such as cell membranes, lysosomes and mitochondria. The effect of APDT on molecular targets such as lipids and proteins has been studied. Damage to these molecules such as peroxidation of membrane lipids and oxidation of specific amino acids in proteins, such as histidine, have been characterized. The proteomic approach allowed the identification of proteins that are more easily oxidized and those more resistant to APDT. One of the prerequisites for the use of a novel PS in preclinical APDT studies is the determination of its toxicity profile. Mammalian cell cultures, and well-established animal models such as the micro-crustacean *Daphnia similis* and embryos of the fish *Danio rerio* have been used in toxicity studies. The results, in general, have shown that the most toxic PS to fungi are also the most toxic to the model organisms. The insect model *Galleria mellonella* is being used to evaluate the effects of APDT *in vivo*. Despite the exponential increase in the number of *in vitro* APDT studies, the number of preclinical studies with novel PS is still small, and the use of APDT in clinical practice remains restricted to the treatment of localized mycoses caused by *Candida* and *Trichophyton* species. However, given the lack of antioxidant-type resistance mechanisms among fungal pathogens, it is predicted, for example, that simple, well-used PS such as methylene blue will be as highly effective against emerging pathogens, such as the currently problematic *Candida auris*, as it is against *C. albicans*. This approach is therefore recommended in the absence of useful conventional therapy. Acknowledgement: FAPESP and CNPq.

> IL106. Invited Lecture
Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

ANTIMICROBIAL PHOTODYNAMIC THERAPY FOR TREATMENT OF ORAL BIOFILM ASSOCIATED DISEASES - WHERE WE ARE

Authors: Håkon Valen[NIOM]
Presenting Author: Håkon Valen
1) Nordic Institute of Dental Materials

Bacteria in the oral cavity lives primarily in polymicrobial biofilms. Oral mature biofilms may contain several hundred different bacterial species, and over 600 different species have been shown to colonize the oral cavity. Bacteria in biofilms have different properties compared to their planktonic, free-floating counterpart.

Dental caries and periodontitis represent the most prevalent diseases globally. Bacteria are necessary etiological agents for dental caries and periodontitis, and develops after dysbiosis, change in the composition, of the microbiota. Bacteria may also invade the pulpal space of teeth and cause endodontic infections. In addition, bacteria may colonize and form biofilm on materials introduced into the oral cavity to restore oral function and aesthetics. Bacteria may colonize dental implant surfaces and cause peri-implant diseases, or colonize denture surfaces which may lead to denture stomatitis. All these diseases are caused by microorganism growing in biofilms. The biofilm may be located in anatomical areas that are difficult to access, such as deep periodontal pockets or inside the root canal system inside a tooth.

Living in a biofilm may facilitate nutritional cooperation, cell signalling and horizontal gene transfer. Bacteria in biofilms are less sensitive to antibacterial compounds compared to their planktonic counterparts. This property of biofilms, challenge dentist in their daily work, and may explain why many agents show promising results *in vitro*, but are less efficacious *in vivo*

In light of the high prevalence and cost related to prevention and treatment of oral biofilm-associated diseases, there is need to develop novel agents, technologies and methods to prevent and combat biofilms that comply with health, without adverse effects. May antibacterial photodynamic therapy be an adjunctive treatment for oral biofilm associated diseases ? This presentation will discuss opportunities and challenges for antibacterial photodynamic therapy treatment of oral biofilm associated diseases in dentistry.

> IL107. Invited Lecture
Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

GENERAL FACTORS THAT DETERMINE BACTERIAL SUSCEPTIBILITY TO ANTIMICROBIAL PHOTODYNAMIC INACTIVATION.

Authors: Joanna Nakonieczna[1]
Presenting Author: Joanna Nakonieczna
1) Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk

Photodynamic inactivation of microorganisms (aPDI) is a potentially good method to destroy antibiotic resistant microbial isolates. Aplication of an exogenous photosensitizer or irradiation of microbial cells already equipped with endogenous photosensitizers makes aPDI a convenient tool for the treatment of infections whenever technical delivery of light is possible. Currently, however, most of the research is performed on *in vitro* models presenting a wide repertoire of the efficacy of aPDI, depending on a photosensitizer used, targeted microorganism, light delivery system. It has been several years since our group started to search for some mechanisms underlying various response to photodynamic inactivation of microorganisms. The ultimate goal was and still is to identify and/or characterize molecular features that drive the efficacy of antimicrobial photodynamic inactivation. For this purpose, we have examined several genetic and biochemical traits, including the presence of particular genetic elements, protein activity, cellular membrane content, and its physical properties, localization of a photosensitizer, with the result that some of them are important while others do not seem to play a key role in the aPDI process. During my presentation, I would like to provide an overview of the factors examined so far that contributed to aPDI process at the cellular level. I would like to challenge a question can one indicate the general pattern of molecular characteristics of the efficacy of aPDI? Or is the photosensitizer-specific pattern of molecular characteritics of aPDI efficacy more likely to occur?

In our work, we used techniques such as DNA genotyping, RNA quantification, UV-Vis spectroscopy of endogenous photosensitizers, proteomics and lipidomics of bacterial cells the results obtained in our research group will be presented and discussed in comparison to the published literature data on the presented issue.

> IL108. Invited Lecture
Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

MUTANTS OF E. COLI, WHICH LACK ANTIOXIDANT ENZYMES, ARE MORE SUSCEPTIBLE TOWARDS TYPE-1 MECHANISM OF ACTION OF PHOTOANTIMICROBIALS COMPARED TO TYPE-2 PHOTOANTIMICROBIALS

Authors: Pouriya Faraj Tabrizi[1], Sara Wennige[1], Tim Maisch[1]
Presenting Author: Tim Maisch
1) Department of Dermatology, University Hospital Regensburg, Germany

Introduction
The photodynamic antimicrobial process is a multi-target method to inactivate pathogenic microorganisms by exciting a photoantimicrobial agent with visible light of appropriate wavelength in the presence of molecular oxygen ($^3O_2$). There are two major pathways by which reactive oxygen species (ROS) are produced. In type-1 reactions, radicals such as superoxide (O2•−) and hydroxyl radicals (•OH) are generated by electron transfer. In type-2 reactions, highly reactive singlet oxygen ($^1O_2$) is produced by direct energy transfer.

Methods
This study investigated the efficiency of the photodynamic antimicrobial process in *Escherichia coli* wild type (EC WT) and the mutant *Escherichia coli* PN134 (EC PN134) which is not able to produce SOD A and SOD B, by means of two different photoantimicrobials from different chemical classes with different $^1O_2$ quantum yields: methylene blue (MB) and 5,10,15,20-tetrakis(1-methyl-4-pyridinio)porphyrin tetra(*p*-toluenesulfonate) (TMPyP).

Results
Mutants, who lack antioxidant enzymes, were particularly susceptible towards type-1 reactions. When using light-activated MB, quenching agents such as superoxide dismutase (SOD) and catalase (CAT) were sufficient for protecting both the wild type and the mutant, whereas they were not able to prevent bacterial killing sufficiently using light-activated TMPyP.

Conclusion
The susceptibility of EC PN134 and EC WT differed towards photodynamic inactivation via the type-1 mechanism of action. Thus, already existing defense mechanisms against ROS in bacteria might influence the susceptibility against type-1 photodynamic mechanism of action, while this was not the case using type-2 photoantimicrobials.

Conflicts of interest
Not given

> OC043. Oral Communication
Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

ANTIMICROBIAL PHOTODYNAMIC INACTIVATION AND ANTIMICROBIAL BLUE LIGHT: SUCCESSFUL ANTIBIOTIC ADJUVANT FOR THE ERADICATION OF EXTENSIVELY DRUG-RESISTANT PATHOGENS AND POSSIBLE TOLERANCE DEVELOPMENT.

Authors: Mariusz Grinholc[1], Agata Wozniak[1], Aleksandra Rapacka-Zdonczyk[1]
Presenting Author: Mariusz Grinholc
1) Laboratory of Molecular Diagnostics, Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Gdansk, Poland.

The worldwide emergence of extensively drug resistant pathogens (XDR) has reduced the number of antimicrobials that exert high bactericidal activity against this pathogen. This is the reason why many scientists are focusing on investigations concerning novel nonantibiotic strategies such as antimicrobial photodynamic inactivation (aPDI) or the use of antimicrobial blue light (aBL). Therefore, the aim of the current study was to screen for antimicrobial synergies of routinely used antibiotics and phototherapies, including both aPDI involving exogenously administered photosensitizing molecules, and aBL, involving excitation of endogenously produced photoactive compounds. The synergy testing was performed in accordance with antimicrobial susceptibility testing (AST) standards, including various methodological approaches, i.e., antibiotic diffusion tests, checkerboard assays, CFU counting and the evaluation of postantibiotic effects (PAEs). We report that combining antimicrobials and aPDI/aBL treatment led to a new strategy that overcomes drug resistance in XDR microorganisms rendering these pathogens susceptible to various categories of antibiotics.

In addition, aPDI and aBL are considered low-risk treatments for the development of bacterial resistance or tolerance due to their multitargeted activity. We assessed the development of *Staphylococcus aureus* tolerance to these phototreatments. Reference *S. aureus* was subjected to 15 cycles of both sub-lethal aPDI and aBL and demonstrated substantial aPDI/aBL tolerance development and tolerance stability after 5 cycles of subculturing without aPDI/aBL exposure. In addition, a rifampicin-resistant (RIF^R) mutant selection assay showed an increased mutation rate upon sub-lethal phototreatments, indicating that the increased aPDI/aBL tolerance may result from accumulated mutations. Moreover, qRT-PCR analysis following sub-lethal phototreatments demonstrated increased expression of *umuC*, which encodes stress-responsive error-prone DNA polymerase V, an enzyme that increases the rate of mutation.

The obtained results indicate that aPDI/aBL leads to successful eradication of XDR pathogens when combined with sub-MIC antimicrobials; however, microbes may develop stable tolerance to studied phototreatments upon sub-lethal aPDI/aBL exposure; thus, the risk of tolerance development should be considered significant when designing aPDI/aBL protocols for infection treatments *in vitro* and in clinical settings.

Acknowledgement
Work was supported by grant no. NCN 2015/19/B/NZ7/02487.

> OC044. Oral Communication
Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

CONTROLLED FORMATION AND ANTIBACTERIAL ACTIVITY OF PHTHALOCYANINE-BASED NANODOTS

Authors: Anzhela Galstyan[1]
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Introduction

Antimicrobial photodynamic therapy (aPDT) is considered as to be a promising alternative for the treatment of bacterial infections. Various photosensitizing agents (PS) able to convert (near)infrared light to reactive oxygen species have been developed and used in antibacterial applications.[1-3] Most reasonable design strategy has been suggested to avoid self-assembly of PS due to the efficacy limitation. Objectives of this study were focused on the synthesis, photophysical characterization and photobactericidal efficacy of phthalocyanine-based photosensitizers and their photoactive self-assembled forms.

Methods

Planktonic cultures and biofilms of Gram-negative and Gram-positive bacterial strains were used in our studies. The results obtained from the colony-forming unit test revealed that the association of 1-10 μM PS with near-infrared light was able to significantly reduce the microbial viable counts also in self-assembled form. TEM measurements show that bacterial membrane is disrupted. XTT cell viability assay and Live/Dead staining were used for quantification of the bacterial biofilms.

Results and Discussion

Commonly inherent hydrophobicity of phthalocyanine derivatives renders them only active in disaggregated state. Degree of electronic interactions depends on the torsional angle of two π-conjugated molecules. So far only few examples of photoactive aggregates of Pcs have been published showing that balance between inactive H-aggregate and active J-aggregate is highly dependent on the electronic interactions between the components (Figure 1). Our results suggest that aggregation induced enhancement of phototoxicity is highly dependent from the metal center and substitution pattern of phthalocyanine core.

Acknowledgements

Financial support from DFG (GA 2362/2-1), Fonds der Chemischen Industrie, COST action “Antimicrobial coating innovations” is gratefully acknowledged.

References

1 A. Galstyan,* R. Schiller, U. Dobrindt, *Angew. Chem. Int. Ed.* **2017**, 56, 10362–10366; *Angew. Chem.* **2017**, 129, 10498–10502.
2 A. Galstyan,* J. Putze, U. Dobrindt, *Chem. Eur. J.* **2018**, *24,* 1178-1186.
3 A. Galstyan, U. Dobrindt, *J. Mater. Chem. B*, **2018**, *6*, 4630–4637.

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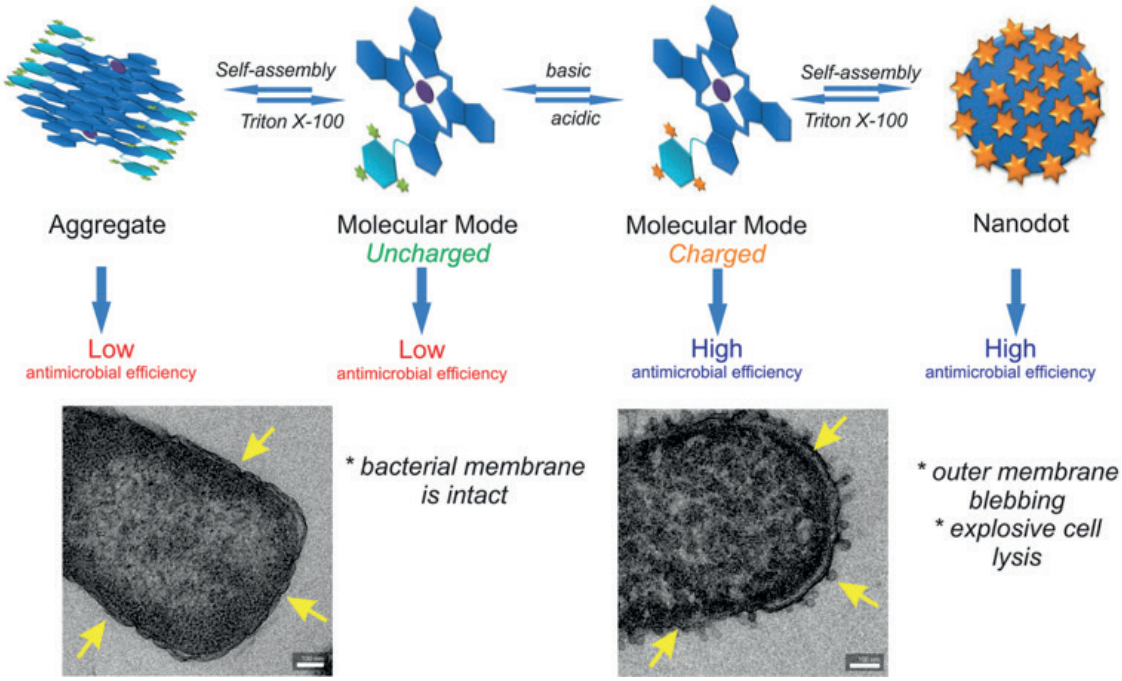
Self-assembly
Triton X-100
basic
acidic
Self-assembly
Triton X-100
Aggregate
Molecular Mode
Uncharged
Molecular Mode
Charged
Nanodot
Low
antimicrobial efficiency
Low
antimicrobial efficiency
High
antimicrobial efficiency
High
antimicrobial efficiency
* bacterial membrane is intact
* outer membrane blebbing
* explosive cell lysis

> P054. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

MODELLING OF DYNAMIC PROPERTIES OF PHOTOINACTIVATION PROCESS

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Researchers working with photodynamic inactivation of bacteria during their workflow try to establish optimal balance between photosensitizer concentration, time of irradiation and light intensity. Usually they try to minimalize concentration of photosensitizer to avoid toxicity and focus on adjusting the irradiation parameters. Main goal is to determine conditions allowing for maximal decrease in bacterial viability. To introduce examined approach into clinic it is necessary to assess also its cyto- and phototoxicity.

In this work we propose a novel approach for photoinactivation studies. We performed a series of experiments to determine viability of Streptococcus agalactiae cells treated with Rose Bengal irradiated with LED lamp of 515nm wavelength. We checked how the bacterial viability change depending on time of irradiation for 12 time points and 4 different values of light intensity. Collected data allowed us to create a model of dynamic properties of photoinactivation process. We determined time constant of transmittance which depends on light power. Created model allows us to predict with a certain probability time of irradiation required for desired effectiveness of photoinactivation.

We intend to create a similar model for phototoxicity against human keratinocytes of applied photoinactivation. Obtained data should allow to determine optimal irradiation parameters which will result in maximal decrease in bacterial viability while preserving viability of human cells. This novel approach may in future contribute for faster implementation of in vitro results into a clinical practise.

This work was supported by the National Science Centre grant no. 2016/23/B/NZ7/03236

> P055. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

THE EFFECT OF PHOTOOXIDATIVE STRESS ON THE EXPRESSION OF GENES ENCODING VIRULENCE FACTORS PRODUCING BY STAPHYLOCOCCUS AUREUS

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Staphylococcus aureus strains produce a wide range of virulence factors, which contribute to its pathogenicity. This group of factors includes toxins, characterized by stability in high temperature, low pH or against proteolytic digestion. What is more, staphylococcal toxins contribute to the development of many diseases, including toxic shock syndrome or food poisoning. It must be emphasized, that a wide range of toxins possess superantigenic properties. Unlike classical antigens, superantigens (SAgs) bind as intact proteins to the T-cell receptor and major histocompatibility complex II molecules (MHC II) outside their binding side. Superantigens are triggering large numbers of T-cells to produce massive amounts of inflammatory cytokines[1]. According to the current literature, superantigens can act as aggravating factors in the inflammation process in atopic dermatitis patients. Furthermore, steroid-resistant atopic dermatitis patients produce a significantly higher amount of superantigens than in the normal population of atopic dermatitis patients. Resistance to this group of drugs can exacerbate the course of atopic dermatitis[2].

The presented research focused on *Staphylococcus aureus* strains producing five toxins: *sea*, *seb*, *sec*, *sed* and *tsst-1*. Two combinations of photodynamic inactivation experiments were used: rose bengal (RB) activated with green light (λ_{max}=515 nm) and new methylene blue (NMB) activated with red light (λ_{max}=632 nm). In the PDI process, *S. aureus* strains were treated under sub-lethal conditions. RNA samples were collected after 20 and 40 minutes the irradiation.

In the qPCR technique, five reference genes: *16S rRNA*, *fabD*, *gmk*, *pyk*, *tpiA* were tested in order to select the most stable gene in the studied experimental conditions. For all of the primers pairs (both genes of interest and reference), standard curves were performed with a 5-fold dilution of cDNA. Melting curves were carried out to exclude contaminations and primer-dimer formation. Reference genes stability were evaluated using three software programs: BestKeeper, geNorm, and NormFinder.

The presented research indicates the photooxidative stress effect on the expression level of genes encoding the staphylococcal virulence factors. Moreover, the influence of the photosensitizers or light itself on the expression of genes encoding specific toxins have been likewise determined.

Acknowledgements
The work is supported by the National Science Centre grant no. 2017/27/B/NZ7/02323 (JN).

References:

[1] Fink, P.J., Matis, L.A., McElligott, D.L., Bookman, M., Hedrick, S.M., 1986. Correlations between T-cell specificity and the structure of the antigen receptor. Nature 321, 219–226.
[2] Schlievert P. M., Case L. C., Strandberg K. L., Abrams B. B., Superantigen Profile of Staphylococcus aureus isolates from patients with Steroid-Resistant Atopic Dermatitis, 2008, Clin Infect Dis, 46(10): 1562-1567

> P056. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

EXPLORATION OF NOVEL FUNCTIONALIZATION REACTIONS FOR NATURALLY OCCURRING PORPHYRINS

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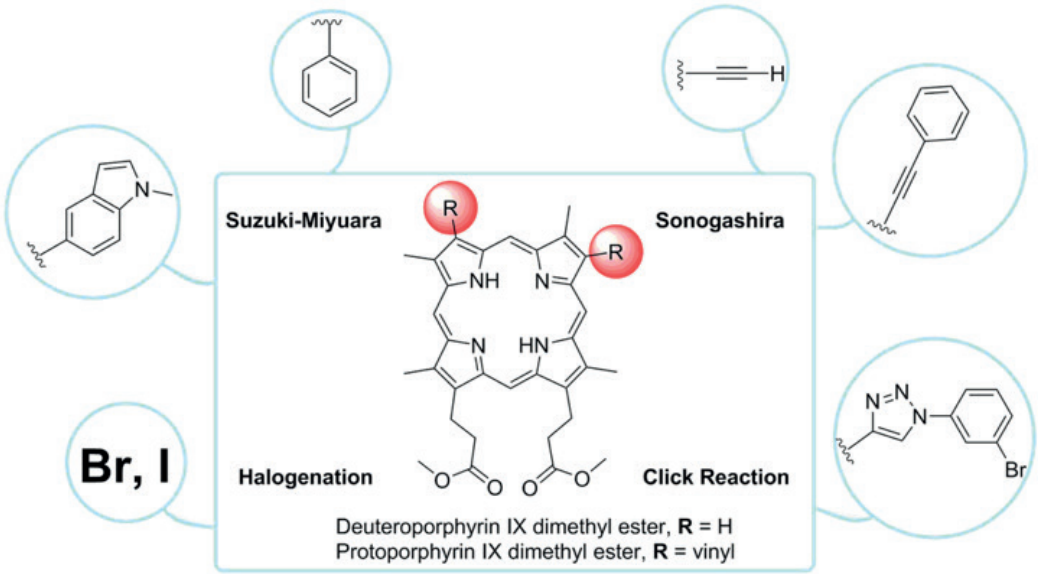
Heme is a complex of protoporphyrin IX with iron and acts as the prosthetic group of hemoproteins. These proteins have a wide range of essential functions in nature, such as oxygen storage and transport, as well as electron transport and catalysis.[1] Recent studies report on the modification of natural porphyrins and their derivatives to alter their catalytic activity. Heme analogs with non-natural metals can catalyze CC bond formation reactions and abiotic derivatives of coenzyme B_{12} can inhibit the *in vitro* catalytic activity of B_{12}-dependent enzymes.[2] These findings suggest that if the cellular uptake of natural porphyrin analogs can be achieved it opens up the possibility to administer porphyrins that have been modified to perform a specific function in cells, e.g. to act as intracellular bio-probes and therapeutic catalysts. In addition, deuteroporphyrin IX derivatives have been shown to function as efficient anti-bacterial PDT agents against Gram-positive and -negative bacteria.[3]

The presented work focuses on novel synthetic modifications of proto- and deuteroporphyrin IX dimethyl esters by palladium-catalyzed cross coupling reactions. Firstly, protocols for halogenations of the vinyl groups of protoporphyrin, respectively the free *β*positions of deuteroporphyrin, were optimized. These sites were further functionalized by Suzuki-Miyaura borylation, Suzuki and Sonogashira cross coupling as well as Click reactions, giving rise to a library of abiotic porphyrins. Among the synthesised derivatives were deuteroporphyrin analogs carrying amine moieties, which, after quaternization of the amino groups, could potentially be used in antimicrobial PDT. Furthermore, the synthesis of protoporphyrin-BODIPY conjugates as bio-imaging tools was investigated. These novel methods for functionalization of natural porphyrins[4] will allow for easy synthesis of biologically relevant porphyrin derivatives and the development of new photosensitizers based on key structural parts of the natural heme molecular framework.

References:

[1] P. Ponka, *Am. J. Med. Sci.* **1999**, 318, 241−256. [2] H. M. Key, P. Dydio, D. S. Clark and J. F. Hartwig, *Nature* **2016**, 534, 534−537; F. J. Widner, A. D. Lawrence, E. Deery, D. Heldt, S. Frank, K. G. Gruber, K. Wurst, M. J. Warren and B. Kräutler, *Angew. Chem. Int. Ed.* **2016**, 55, 11281−11286. [3] Y. Nitzan, M. Gutterman, Z. Malik and B. Ehrenberg, *Photochem. Photobiol.* **1992**, 55, 89–96. [4] M. O. Senge, *Chem. Commun.* **2011**, *47*, 1943–1960.



> P057. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

PDI SENSITISER COMBINED WITH AN ANTIMICROBIAL PEPTIDE: TOWARDS A MOLECULAR TARGETED ANTIBACTERIAL AGENT

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Presenting Author: Charly Gourlot
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The emergence of multidrug-resistant bacteria and fungi towards antibiotics constitutes a major health problem responsible for million deaths every year all over the world.[1] An alternative to antibiotics consists in the use of the PDI (PhotoDynamic Inactivation) to destroy drug-resistant bacteria without inducing new resistances.[2] This technique consists in the activation of a photosensitiser (PS) that targets bacteria with light of suitable wavelength to produce reactive oxygen species. For a selective and efficient PDI treatment, one approach is to conjugate the PS with an AMP (AntiMicrobial Peptide). Such association has shown encouraging results on gram-positive and gram-negative bacteria and seems promising for the future.[3]

Our goal is to develop new photoactivable antibacterial drugs, which combine PS with AMP. An antibacterial agent composed of a porphyrin linked to PGLa has been synthesized (Figure 1). The PS has to be activated inside the therapeutic window (700-1000 nm) to prevent photodamage to healthy tissue and should have a high quantum yield of singlet oxygen. Therefore, a porphyrin with extended pi-conjugated system over a π-acceptor unit was selected. It was associated to a cationic peptide, PGLa, a peptide that has shown good membrane disrupter activity.[4] The synthesis as well as the preliminary studies on bacteria of the PS-AMP conjugate will be presented.

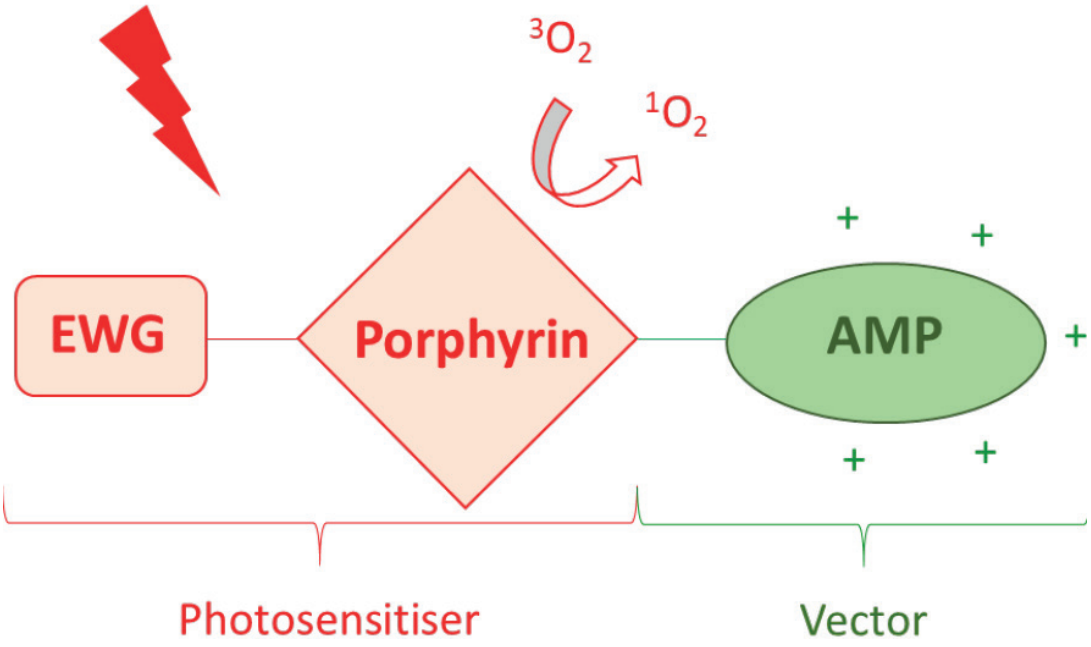

Figure 1: Antimicrobial agent combining a porphyrinic photosensitiser and an AMP.

References

[1] A. Regiel-Futyra, J. M. Dabrowski, O. Mazuryk, K. Spiewak, A. Kyziol, B. Pucelik, M. Brindell, G. Stochel, *Coord. Chem. Rev.*, **2017**, *351*, 76-117.
[2] L. Jiang, C. R. R. Gan, J. Gao, X. J. Loh, *Small*, **2016**, *12*, 3609-3644.
[3] F. Biscaglia, M. Gobbo, *J. Pept. Sci.*, **2018**, *110*.
[4] B. Bechinger, *J. Pept. Sci.*, **2015**, *21*, 346-355.

> P058. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

PHOTOSTABILITY OF AN ANIONIC PORPHYRIN IN NATURAL DEEP EUTECTIC SOLVENTS (NADES)

Authors: Krister Gjestvang Grønlien[1], Kristine Opsvik Wikene[1], Ellen Bruzell[2], Håkon Valen[2], Hanne Hjorth Tønnesen[1]
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Natural deep eutectic solvents (NADES) is a group of eutectics that is based solely on environmental-friendly ("Green") components. These solvents have unique solubilizing properties; i.e. they are more polar than water but can still dissolve highly lipophilic compounds such as aromatic heterocycles like porphyrins. Further, certain NADES possess antimicrobial properties and increase the phototoxicity of porphyrins against e.g. Gram -negative bacteria. Selected NADES are thereby suitable as solvents in antimicrobial photodynamic therapy (aPDT). We have previously demonstrated that NADES can potentiate the phototoxic effect of the anionic porphyrin TCPP (4, 4', 4'', 4'''-(porphine-5, 10, 15, 20-tetrayl) tetrakis benzoic acid) towards Gram-negative bacteria (1). The potentiating effect was dependent on the type of NADES. The present work includes a photostability study on TCPP in two relevant NADES of different pH but with comparable polarity and viscosity; choline chloride - xylitol (5:1) and malic acid – glucose – fructose (1:1:1). The photodegradation was studied in undiluted NADES and in NADES diluted (1:1) in MilliQ water or PBS, respectively. The following parameters were investigated: reaction order, reaction rate constant, shelf-life, absorption and emission characteristics, and the relative potential to generate singlet oxygen. The results demonstrate that the photoreactivity of TCPP was dependent on the type of NADES, the dilution factor and type of dilution medium. This emphasizes that a small modification of the formulation; e.g. change in dilution medium from water to PBS, can have an impact on the photochemical behavior of the drug molecule. The observed effects constitute essential information in the development of consumer products.

Reference

Wikene, K.O., Rukke, H.V, Bruzell, E, Tønnesen, H.H., Physicochemical characterisation and antimicrobial phototoxicity of an anionic porphyrin in natural deep eutectic solvents, Eur. J. Pharm. Biopharm., 105, 75-84 (2016)

> P059. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

PROTECTIVE ROLE OF THE STAPHYLOXANTHIN PIGMENT AGAINST PHOTOINACTIVATION OF STAPHYLOCOCCUS AUREUS

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Background

Staphylococcus aureus is the causative agent of a diverse array of acute and chronic infections. This pathogen has acquired resistance to virtually all of the antimicrobial agents available, and in recent years, the worldwide emergence of multiresistant clones in hospitals and communities has spurred significant concern. *S. aureus* produces a yellowish-orange pigment, named staphyloxanthin (STX), which is the product of carotenoid biosynthesis pathway. Because of the molecular structure of the pigment, STX could act as an endogen photosensitizer in photoinactivation with visible light treatment. However, STX could protect to *S. aureus* from the photoinactivation due to its antioxidant properties.

Objective

To study the response of *S. aureus* to photoinactivation treatment mediated by toluidine blue in the presence or absence of STX.

Methods

S. aureus laboratory strains SH1000, RN6390 and RN6911 and the clinical isolates Sa14 and Sa14P were employed. Methanolic extracts of the pigment from bacterial suspensions with equal optical density (OD) were quantified spectrophotometrically at 450 nm. To photoinactivate *S. aureus*, the bacterial suspensions were illuminated in the presence of toluidine blue (50 μM) employing a non-coherent light source in the presence or absence of STX added exogenously. Then, the viable cells were quantified by plating an aliquot of serial dilutions on trypticase soy agar (TSA). The response to oxidative stress with H_2O_2 was determined immediately after photoinactivation treatment.

Results

The Abs450 of STX extracts were as follows: SH1000 (0.468±0.045), RN6390 (0.164±0.096), RN6011 (0.094±0.02), Sa14 (0.205±0.033) and Sa14P (0.450±0.123). The pigment production was similar up to 14 days of TSA plate growth. Absorption spectra of the methanolic extracts were similar between SH1000 and Sa14P strains and also between Sa14 and RN6390 respectively. For SH1000, RN6390 and Sa14, the photoinactivation mediated by toluidine blue reduced the number of viable bacteria by 4, 5 and 6 orders of magnitude respectively, as compared to non- irradiated controls. For Sa14P, the same treatment reduced 6 orders of magnitude the amount of viable bacteria. The addition of exogenous STX reduced the photoinactivation degree of RN6911 (white colonies) or SH1000 (orange colonies) to 1 order of magnitude in both strains. After photoinactivation treatment, all viable bacterial cells incubated with exogenous STX resisted the oxidative stress of H_2O_2, exhibiting similar number of CFU/ml as compared to the non-H_2O_2 treated control. In contrast, exposure to H_2O_2 killed the few viable bacterial cells detected after photoinactivation treatment in the absence of exogenous STX addition.

Conclusions

The pigment STX either endogenously or exogenously protects *S. aureus* against photoinactivation mediated by toluidine blue. Antioxidant properties of STX could be responsible of its protective role.

> P060. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

PHOTODYNAMIC INACTIVATION OF CANDIDA ALBICANS IN BLOOD

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Blood is an essential resource but is yet a source of microbial infections transmission. Consequently, the ability to disinfect blood and its derivatives has assumed great importance. Currently, the most effective method for inactivating microorganisms, which can be only used in plasma or protein concentrates, is the combined use of a solvent tri(n-butyl) phosphate and a detergent Tween 80 mixture. However, these chemicals must be removed after treatment because they are harmful to the membranes of erythrocytes and platelets of blood receptors. Antimicrobial Photodynamic Therapy (aPDT) has been suggested as an alternative technique to blood disinfection. Methylene blue (MB), psoralen and riboflavin are already approved photosensitizers (PS) in some countries to disinfect plasma, but there is no aPDT approved application for whole blood, concentrated platelets or erythrocytes. The aim of this study was to evaluate the effectiveness of aPDT to inactivate *Candida albicans*, a microorganism frequently involved in bloodstream infections. For that, several cationic porphyrin derivatives were used to photoinactivate *C. albicans* in phosphate buffered saline (PBS), plasma and whole blood. Once MB is the mostly used PS to disinfect plasma, its efficacy was also evaluated for comparison. Samples and controls were exposed to white light (400-800 nm) at an irradiance of 2.5 mW/cm^2 and 150 mW/cm^2, respectively, for PBS and whole blood/plasma, for 270 min. All the tested cationic porphyrins were effective to photoinactivate *C. albicans* in PBS. In plasma, the photoinactivation was lower (reduction of 3 logs after 270 min of treatment), being the Tri-Py(+)-M-PF the most effective PS. In the whole blood, this cationic PS at 10 μM had promoted a small decrease in the survival of *C. albicans* (reduction of about 1 log). The photoinactivation of *C. albicans* using MB at 5 μM in PBS and 10 μM in plasma was 0.8 log and 0.7 log, respectively, which was significantly less effective than the cationic porphyrins. In whole blood the MB was not able to inactivate *C. albicans*. The results indicate that aPDT using cationic porphyrins seems to be a promising approach for the photoinactivation of *C. albicans* in plasma, but more studies are needed to improve their inactivation in the whole blood.

Acknowledgements

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> P061. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

ANTIMICROBIAL EFFICACY AND MECHANISM OF ACTION OF PHENALEN-1-ONE MEDIATED ANTIMICROBIAL PHOTODYNAMIC THERAPY IN BACTERIAL BIOFILMS

Authors: Denise Muehler[1], Sercan Keceli[1], Christina Rupp[1], Karl-Anton Hiller[1], Tim Maisch[2], Wolfgang Buchalla[1], Fabian Cieplik[1]
Presenting Author: Denise Muehler
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Introduction

In view of increasing resistance against antibiotics and antiseptics, antimicrobial photodynamic therapy (aPDT) is a promising alternative in dentistry. The aim of this study was to evaluate the antimicrobial efficacy of aPDT with the phenalene-1-one derivative SAPYR in bacterial monospecies biofilms of *Actinomyces naeslundii*, *Streptococcus mutans* and *Escherichia coli*. Furthermore, the effect of aPDT on membrane integrity, metabolic activity and formation of reactive oxygen species (ROS) was investigated.

Methods

Monospecies biofilms (*Actinomyces naeslundii* DSM43013; *Streptococcus mutans* DSM20523 and *Escherichia coli* DSM1103) were cultured under aerobic conditions for 48 h followed by treatment with the photosensitizer SAPYR at various concentrations (50, 100, 500 µM) at different incubation times (5, 10, 20, 30 min) and subsequent irradiation for 10 min (Waldmann PIB 3000; I_{em} = 360-600 nm; 50 mW/cm²; 30 J/cm²). Control samples were treated with dH_2O and kept in dark for the same time. Antimicrobial efficacy was evaluated by CFU assay. The cell membrane integrity as a possible target structure after aPDT was investigated with flow cytometry using SYBR Green and propidium iodide. Metabolic activity and formation of ROS were evaluated via fluorometric assays.

Results

SAPYR showed antimicrobial effects (>3log_{10} CFU) on *S. mutans* after 5 min and on *A. naeslundii* after 30 min incubation time with SAPYR. For *E. coli*, CFU reduction was >2log_{10}after 30 min of incubation. Membrane damage upon aPDT could be revealed for *E. coli*, but not for *S. mutans* and *A. naeslundii*. Fluorometric assays showed a reduction in metabolic activity and an increase in formation of ROS in all three species upon aPDT treatment.

Conclusions

After treatment with aPDT, monospecies biofilms cleary showed decreased ability to replicate (CFU assay). However, the mechanism of action regarding membrane damage is apparently different for Gram-negative and Gram-positive bacterial species after treatment with SAPYR. For further understanding of the mechanisms of action it is necessary to verify the results of this study by investigating changes in protein and gen expression after treatment with aPDT.

> P062. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

ANTIMICROBIAL PHOTODYNAMIC ACTIVITY OF MACROPOROUS POLYSTYRENE LOADED WITH ROSE BENGAL

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Introduction

Staphylococcus aureus and *Escherichia coli* are commonly involved in various infections.

Antimicrobial photodynamic therapy (aPDT) can be alternative treatments in which reactive oxygen species (ROS) are generated with light irradiation. Macroporous polystyrene (P_{mp}) containing alkylammonium groups on the surface has been used as a support for photosensitizing molecules.

The aim was to compare the *in vitro* efficacy of aPDT using the P_{mp} loaded with the photosensitizer Rose Bengal (RB) against *S. aureus* and *E. coli.*

Materials and methods

Microbial suspensions containing $>10^7$ cells/mL were prepared. The P_{mp} was obtained from commercial sources (Amberlite™ IRA 900, Sigma-Aldrich). Final loading of RB was 1.5 mg/g of resin. Six groups of samples were prepared: three for the irradiation with 515 nm-LED lamp (5.8 mW/cm^2) and three as controls in the darkness. Five mL of the microbial suspensions were dropped into different RODAC plates and then (I) 200 mg of P_{mp} loaded with RB, or (II) the same amount of control matrix (resin without RB), or (III) no resin were added. The six groups were shaken during the time of the photodynamic treatment.

The antimicrobial effect at different light doses up to a maximum of 200 J/cm^2 was determined by counting the number of colony-forming units (CFU)/mL on blood agar.

Results

P_{mp} loaded with RB achieves a 1 $\log_{10}$ and 2.5 $\log_{10}$ reduction in bacterial growth of *S. aureus* and *E.coli* respectively at the dose of 100 J/cm^2. These reductions are increased until reaching a reduction of 5 $\log_{10}$ and 6 $\log_{10}$ respectively at 200 J/cm^2. The irradiation by itself or the polymeric matrix have no effect on the number of bacteria compared to the initial value.

Discussion

RB is a photosensitizer very effective to photoinactivate Gram positive bacteria. Only cationic photosensitizers are active against Gram negative bacteria and usually require more doses of both photosensitizer and fluence. RB is anionic but P_{mp} is a cationic molecular vehicle. This feature, along with the enhanced porosity of the matrix, could explain why RB supported on this polymer is effective against the Gram negative bacteria in this study.

Conclusions:

P_{mp} loaded with RB has antimicrobial effect against *S. aureus* and *E. coli.*

P_{mp} loaded with RB is more effective in killing *E. coli* than *S. aureus* bacteria.

P_{mp} could enhance the effect of RB-aPDT against Gram negative bacteria.

Continued

Acknowledgements:
AR and YG were funded by the Aragón Government: B10_17R Infectious Diseases of Difficult Diagnosis and Treatment research group and B18_17D Dermatology and Photobiology research group, respectively. The authors thank the IIS Aragón for the GIIS-023.

Conflicts of Interest:
The research reported was conducted in the absence of any commercial or financial relationships that could constitute potential conflicts of interest.

> P063. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

EVALUATION OF THE LEISHMANICIDAL POTENTIAL OF AMPHIPHILIC CHLORINS MEDIATED BY PHOTODYNAMIC THERAPY

Authors: Irwin A.P. LInares[2], Angela M.A. Velásquez[1], Marcia A.S. Graminha[1], Kleber T. Oliveira[3], Janice R. Perussi[2]
Presenting Author: Janice Perussi
1) Universidade Estadual Paulista Julio de Mesquita 2) Universidade de São Paulo 3) Universidade Federal de São Carlos

Leishmaniasis is a parasitic disease that affects approximately 350 million people living in high-risk areas around the world, it is caused by parasitic protozoa of the genus Leishmania transmitted by the insect bite of the subfamily Phlebotominae. Conventional treatments involve pentavalent antimonial drugs that are aggressive and trigger a diversity of side effects. Photodynamic therapy (PDT) is a promising alternative treatment for leishmaniasis especially due to the possibility of a local treatment, without adverse effects. PDT relies on the interaction between a light-sensitive compound (photosensitizer), light and molecular oxygen. The reaction generates singlet oxygen ($^1O_2$) and other cytotoxic reactive oxygen species (ROS) which induce cell death by oxidative stress. This study aimed to evaluate three-chlorin derivatives internalization and the efficacy of PDT on *L. amazonensis*. The confocal microscopy showed that the three-chlorin were located inside the cytosol of the parasites forming agglomerations exhibiting a high fluorescence emission after 2 h incubation and co-localized in the acidic compartments of the parasites labeled with Lysotracker green. Chlorin CHL-OH-A showed the higher mitochondrial activity by colorimetric MTT assay with IC_{50} values of 0.35 and 0.14 μmol L^{-1} with irradiation at 660 nm (6.0 J cm^{-2}) after incubation for 24 and 48 h, respectively. CHL-OH-B and CHL-TRISMA molecules induced a high percentage of apoptotic-like cells (60%) while CHL-OH-A only about 40% caused by a greater oxidative stress in the cell inducing the necrosis mechanism after 24 h of treatment (6.0 Jcm^{-2}). This study showed that these amphiphilic chlorins, and in particular, CHL-OH-A, exhibited a higher leishmanicidal activity in the promostigote forms suggesting that these molecules could be used in PDT to be evaluated against the intracellular amastigote forms that are the clinically relevant forms.

References

1. Linares, I.A.P., et al., *Dyes and pigments*, 145 (2017) 518.
2. Pinto, J. G., et al., *Photodiagnosis and Photodynamic Therapy,* 15 (2016)19.
3. Moritz, M.N.O, et al., *Photodiagnosis and Photodynamic Therapy,*17 (2017) 39.
4. Minodier, P., Parola, P., Travel Medicine and Infectious Disease, 5, (2007) 150.

> P064. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

MULTIPLE SUB-LETHAL ANTIMICROBIAL PHOTODYNAMIC INACTIVATION AND BLUE LIGHT TREATMENT OF *S. AUREUS* LEAD TO TOLERANCE DEVELOPMENT

Authors: Aleksandra Rapacka-Zdonczyk[1], Agata Wozniak[1], Mariusz Grinholc[1]
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Introduction

After almost 100 years after the discovery of the penicillin, future of antibiotic therapy is uncertain. Growing number of multi-drug resistant organisms, especially *Staphylococcus aureus*, forces the alternative therapies development. Photodynamic inactivation (aPDI) and antimicrobial blue light treatment (aBL) are very promising therapeutic, multi-targeting options which causes functional and morphological damages in bacterial cells. aPDI consists of three elements: exogenous photosensitizing agent (PS), appropriate wavelength light and oxygen, aBL is based on endogenous PS.

Aim of the study

The current study was aimed to investigate if repeated exposure to Rose Bengal sub-lethal inactivation (RB-aPDI) and antimicrobial blue light treatment affect susceptibility to those treatments and lead to the tolerance development.

Materials and methods

Reference strain of *S. aureus* US300 (CA-MRSA) was used in the experiments. Irradiation was performed with two LED light sources that emitted blue (λmax 411 nm) and green (λmax 515 nm) light. 15 repeated cycles of sub-lethal photoinactivation was followed by bacteria re-growth overnight. A potential reduction in susceptibility to aPDI/aBL was tested after 5th, 10th and 15th of consecutive cycle at the higher light doses irradiation. Additionally, a potential increases in the mutation rate associated with rifampicin resistance was tested. Also, *S. aureus* bacteria subjected to multiple sub-lethal phototreatments were examined for their susceptibility to selected antimicrobials.

Results

The obtained results demonstrate that multiple sub-lethal phototreatments may lead to *S. aureus* tolerance development. RB-aPDI tolerance development was induced only with the RBaPDI sub-lethal treatment and similarly, aBL tolerance development was induced only with the aBL sub-lethal treatment. Also sensitization to antimicrobial agents, gentamycin and doxycycline was observed. Moreover, the results indicate that, both sub-lethal aPDI and sub-lethal aBL lead to an increased mutation rate in *S. aureus* to rifampicin.

Conclusions

aPDI/aBL-induced DNA damages may be responsible for the genetic alterations that lead to the increased tolerance of *S. aureus* to the photodynamic inactivation and increased susceptibility to the antibiotic treatment.

The work was supported by the National Science Centre (Poland) grant no. 2015/19/B/NZ7/02487

> P065. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

EFFECTS OF BLUE LIGHT ON PSEUDOMONAS AERUGINOSA BIOFILM FORMATION AND ERADICATION

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Introduction

The opportunistic pathogen *Pseudomonas aeruginosa* can cause severe nosocomial infections in different body districts, including wounds, ulcers and urinary tract. This microorganism takes advantage of a combination of resistance mechanisms to overcome the action of antimicrobials and, as a result, infections become difficult to treat, especially when *P. aeruginosa* grows as biofilm [1]. Within recent anti-*Pseudomonas* approaches, antimicrobial Blue Light Therapy (aBLT) gained increasing interest. aBLT is based on the effect of visible light, particularly in the region from 390 to 500 nm, to control the bacterial growth and biofilm formation of a broad-spectrum of pathogens, including bacteria, yeasts and fungi [2]. The mechanism of action is not fully understood. It has been hypothesized that endogenous photosensitizers may induce photo-oxidative stress upon irradiation causing photo-oxidation of microbial macromolecules and cellular death, as a consequence [3].

Methods

In this study, blue light at 410 and 455 nm were used to inhibit and/or eradicate biofilm of *P. aeruginosa* PAO1, chosen as model microorganism. A multi-well plate was used as *in vitro* setup. Crystal violet staining of adherent biofilm, combined with cell viability of planktonic and sessile populations, permitted to evaluate the effect of blue light on cells and matrix. Confocal microscopy analyses have been also performed to evaluate the efficacy of aBL.

Results

Upon increasing radiant exposures, blue light at 410 nm successfully inhibited biofilm formation of *P. aeruginosa* PAO1, causing a significant decrease in adherent biomass and cell viability of adherent and planktonic phases. Blue light at 455 nm showed a very good inhibitory effect. Fifteen *P. aeruginosa* strains isolated from catheters-associated urinary tract infections, characterized by a different ability to form biofilm, were sensitive to aBL. Moreover, blue light at 410 nm was also active in eradicating young and old biofilms of PAO1 strain. Interestingly, blue light seems to affect the ability to form matrix. Further investigations are needed to evaluate how blue light damages biofilm machinery.

Conclusions

Blue light at 410 nm is effective in inhibiting and eradicating *P. aeruginosa* biofilm in a dose-light dependent manner. This approach could be exploited in different applications in which *P. aeruginosa* growth control is needed, such as clinical, environmental and industrial fields.

References

[1] Azam, Mohd W., and Asad U. Khan. “Updates on the pathogenicity status of Pseudomonas aeruginosa.” *Drug discovery today* (2018).
[2] Hamblin, Michael R., and Heidi Abrahamse. “Can light-based approaches overcome antimicrobial resistance?” *Drug Development Research* 80.1 (2019): 48-67.
[3] Wang, Yucheng, et al. “Antimicrobial blue light inactivation of pathogenic microbes: State of the art.” *Drug Resistance Updates*33 (2017): 1-22.

> P066. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

PHOTODYNAMIC INACTIVATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ON SKIN USING A PORPHYRINIC FORMULATION AS PHOTOSENSITIZER AND POTASSIUM IODIDE

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Staphylococcus aureus is the leading cause of a wide range of severe clinical infections like skin and soft tissue infections. This bacterium is capable to acquire resistance to the usually used antibiotics as typified by methicillin-resistant *Staphylococcus aureus* (MRSA).[1] Antimicrobial Photodynamic Therapy (aPDT) emerged as an alternative treatment for localized infections in response to the ever-growing problem of antibiotic resistance. The combination of aPDT and antibiotics was already reported as an efficient approach to inactivate successfully *S. aureus* (*in vitro* and *ex vivo*).[2] It was demonstrated that using 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin tetra-iodide (Tetra-Py(+)-Me) as photosensitizer (PS), a total inactivation of *S. aureus* after 3 cycles of treatment or after 1 cycle using the combination aPDT and ampicillin is achieved.[2] This porphyrin is one of the constituents of a formulation (FORM), based on a non-separated mixture of 5 cationic meso-tetraarylporphyrins that proved to be effective in aPDT of several bacteria, namely *S. aureus*.[3] FORM has been recognized as an excellent alternative to the highly efficient Tetra-Py(+)-Me since the production costs and time were reduced significantly. aPDT effect can be also potentiated by using a combination of PSs and inorganic salts, such as potassium iodide (KI), that is recognized to increase the aPDT efficiency of some neutral and cationic PSs on a broad-spectrum of microorganisms.[3] The objective of this study was to evaluate the efficacy of FORM with KI to photoinactivate MRSA on skin. For this, pork skin was artificially contaminated with MRSA (*ex vivo*) and treated with FORM or FORM + KI under white light. The aPDT protocol with the combination of FORM and KI was first developed in Phosphate Buffered Saline (PBS, *in vitro*). The results showed that FORM was effective in aPDT of MRSA in PBS, where total inactivation was achieved at a concentration of 5.0 μM. For the combination FORM + KI total inactivation of MRSA was observed using 0.5 μM of FORM. In *ex vivo*, a reduction of ~3 log of MRSA was observed with 50 μM of FORM. In this case KI did not potentiate the FORM efficiency. The results show that aPDT using FORM as PS seems, even without coadjutants, to be a promising therapy for the inactivation of MRSA on skin.

Thanks to University of Aveiro and FCT/MEC for the financial support to QOPNA (FCT UID/QUI/00062/2019) and CESAM (UID/AMB/50017/2019), to FCT/MEC through national funds and to co-funding by the FEDER-Operational Thematic Program for Competitiveness and Internationalization-COMPETE 2020, within the PT2020 Partnership Agreement.

References

1. Tong, SYC et al. Clin Microbiol Rev 2015, 28, 603.
2. Branco, TM et al. Photodiagnosis Photodyn Ther 2018, 2, 285.
3. Vieira, C et al. Front Microbiol 2018, 9, 2665.

> P067. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

LYSINE ANALOGUE OF POLYMYXIN B AS A SIGNIFICANT OPPORTUNITY FOR PHOTODYNAMIC ANTIMICROBIAL CHEMOTHERAPY

Authors: Florent Le Guern[1], Tan-sothea Ouk[1], Catherine Ouk[2], Régis Vanderesse[3], Yves Champavier[2], Emilie Pinault[2], Vincent Sol[1]
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Introduction

Infected wounds are a major cause of hospital-acquired infections. Their management becomes difficult due to the emergence of MultiDrug Resistant bacteria. Looking for new therapeutic approaches is primordial. Among them, Photodynamic Antimicrobial Chemotherapy (PACT) was developed. However, the use of PACT for the treatment of infected wounds requires a specific targeting of PS to bacteria without inducing damages on the skin cells.

In this work, a new antimicrobial peptide conjugate has been synthesized, consisting of a cationic porphyrin covalently attached to a derivative of polymyxin B (PMB), in order to specifically target bacterial cell wall.

Methods

PMB is an antimicrobial peptide, known to target Lipid A from Gram-negative cell wall. A PMB-derived moiety (**1Lys**) was subjected to a primary structural modification in the replacement of four diaminobutyrate residues (L-Dab) with L-Lys residues. A cationic porphyrin (5-(4-aminophenyl)-10,15,20-tri(4-N-methylpyridyl)-21H,23H-porphyrin tetraiodide) has been linked to the polymyxin-derived moiety using a spacer and a thiol-maleimide ‘click” coupling, resulting to a new conjugate (**5Lys**).

Results and Discussion

Bactericidal properties of new synthesized molecules have been evaluated against three bacterial strains (*S.aureus*, *P.aeruginosa*, and *E.coli*) and the results of Minimal Bactericidal Concentration (MBC) were resumed in the table.

As expected, peptide alone (**1Lys**), as well as the peptidic moiety of this new conjugate (**5Lys**), have shown a significant loss of activity in the dark against Gram-negative bacteria, contrary to the original molecules containing L-Dab (compounds **1** and **5**). After light irradiation, the bactericidal activity of **5Lys** was comparable to the one obtained with compound **5**. Flow cytometry analyses have demonstrated that the affinity of the new conjugate (**5Lys**) for bacteria and its ability to weaken bacterial membrane has been preserved.

Conclusions

The structural modification of PMB by replacing L-Dab with L-Lys was done with the aim to eliminate the potential rise of polymyxin-resistant strains. Despite this modification, this new conjugate displayed a strong photobactericidal activity against Gram-positive as well as Gram-negative bacteria.

Acknowledgements

The authors thank the “Conseil Regional du Limousin” (FRANCE) for the financial support

References:

(a) F. Le Guern *et al.*, Bioconj. Chemistry 2017, 28(9): 2493-2506
(b) F. Le Guern *et al.*, ACS Med. Chem. Letters 2018, 9(1): 11–16

Continued

	MBC (μM)					
	S.aureus		*P.aeruginosa*		*E.coli*	
Compounds	Light	Dark	Light	Dark	Light	Dark
1[a]	>50	>50	10.0	10.0	5.0	5.0
5[a]	0.8	-	2.5	10.0	0.5	1.2 - 5.0
1Lys[b]	-	-	>100	>100	50.0	50.0
TMPyP	5.0	-	20.0	-	18.0	-
5Lys[b]	1.2	-	8.0	>100	0.8	25.0

> P068. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

EVALUATION OF HELICOBACTER PYLORI PHOTOINACTIVATION BY USING A NOVEL LED-BASED DEVICE

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The rise of antibiotic resistance is the main cause for the failure of conventional therapy of *H. pylori* infection, which is often associated with severe gastric diseases, including gastric cancer. Antimicrobial PhotoDynamic Therapy (aPDT) could represent a promising therapeutic strategy. In the case of *H. pylori*, aPDT exploits photoactive endogenous porphyrins to induce photokilling [1, 2, 3]. The project *CapsuLight* is aimed at developing an ingestible LED-based robotic pill for minimally invasive intragastric treatment of *H. pylori* infection. In this framework, it is crucial to determine the best illumination parameters to activate the *H. pylori* photosensitizers. This study is aimed at evaluating the photokilling effect on *H. pylori* by using a novel LED-based device [4], developed to perform in *vitro* irradiation tests. Moreover, photodynamic effects on bacterial cell were assessed by Scanning Electron Microscopy (SEM).

Two *H. pylori* strains, ATCC43504 and the virulent ATCC700824, were used. An aliquot of a bacterial suspension was irradiated by means of the LED equipped device at 405, 460, 500 and 630 nm. The photokilling efficacy compared to the dark control was assessed by plating serial dilutions of each sample and by viable counting. Before SEM imaging, irradiated samples were fixed, dehydrated and dried at the critical point.

The exposure to various levels of visible light through the LED-based devices caused a bactericidal effect on both strains of *H. pylori*. Among the tested wavelengths, the 405 nm one was the most efficient in killing, inducing a dose-dependent reduction of bacterial count, compared to the dark control. Irradiated *H. pylori* cells appeared damaged with evident holes on the bacterial surface, likely leading to cell death. Our findings suggest that aPDT could be a valid alternative or adjuvant therapy to conventional antibiotics for *H. pylori* infection.

Acknowledgements

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References

1. Hamblin M. R.et al. *Helicobacter pylori* accumulates photoactive porphyrins and is killed by visible light. Antimicrob Agents Chemother, 2005. 49(7): 2822–2827.
2. Battisti A. et al. Spectroscopic characterization and fluorescence imaging of *Helicobacter pylori* endogenous porphyrins. Biophys Chem. 2017. 229: 19-24.
3. Battisti A. et al. Compositional analysis of endogenous porphyrins from *Helicobacter pylori*. Biophys Chem. 2017. 229: 25-30.
4. Battisti A. et al. Temperature increase inside LED-based illuminators for in vitro aPDT photodamage studies. Results in Physics. 2018. 9: 680:681.

> P069. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

PORPHYCENES AS PHOTOSENSITIZERS FOR PHOTODYNAMIC INACTIVATION OF BACTERIA

Authors: Natalia Masiera[1], Aleksander Gorski[1], Agnieszka Bojarska[2], Iwona Gawryszewska[2], Jacek Waluk[1]
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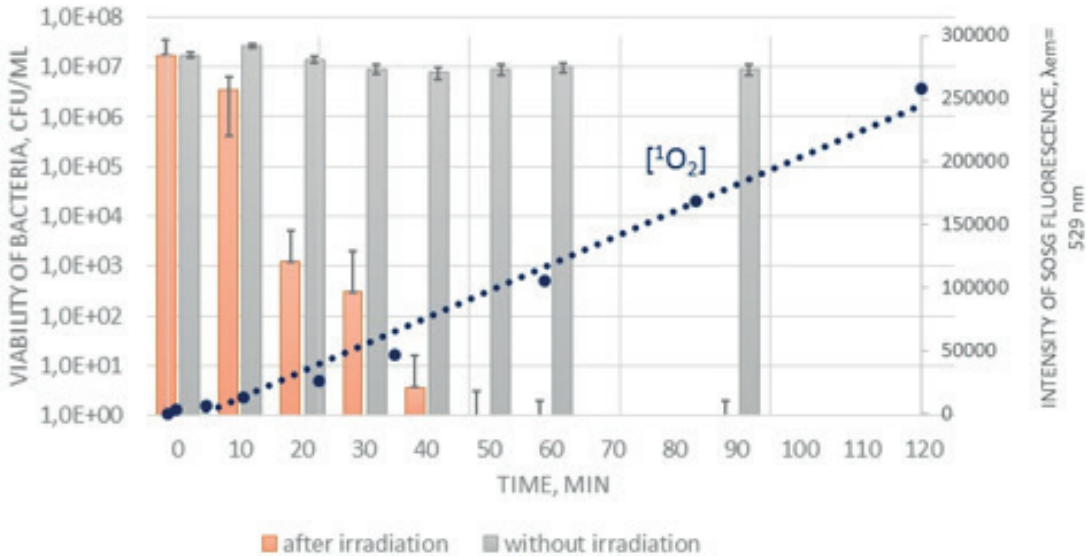
Porphycenes are studied as potential candidates for second generation of photosensitizers. With the high absorption coefficients in the red part of spectrum, they were proven to exhibit significant phototoxic effect against cancer cells [1] and bacteria [2]. A perfect photosensitizer should be characterized by such properties as high molar absorption coefficients in the optical therapeutic window range and high quantum yield of reactive oxygen species (ROS) generation.

Figure 1. Dependence of the viability of E. faecalis bacteria on the time of irradiation of pluronic (3.5 mM) solution samples porphycene(7 μM); overlayed fluorescence intensity of SOSG, corresponding to the concentration of singlet oxygen in the sample.

The subject of present studies is the correlation between the ROS generation and antimicrobial activity for a group of differently substituted porphycenes. One of the aims was the evaluation of singlet oxygen generation quantum yields of selected chromophores in order to assess their photosensitizing abilities. The second area of interest was the dependence of their performance in *in vitro* PDI trials with respect to the differences in the molecular structure of the porphycene-core compound. The results of research show that the quantum yields of singlet oxygen generation are comparable among the studied group of compounds, whereas the PDI efficiency differs significantly depending on the substituents of porphycene core. The structure of the molecule is therefore critical for the interaction with the bacteria cells.

References

[1] J. C. Stockert, M. Cañete, A. Juarranz, A. Villanueva, R. W. Horobin, J. I. Borrell, J. Teixidó, and S. Nonell, “Porphycenes: facts and prospects in photodynamic therapy of cancer,” *Curr. Med. Chem.*, vol. 14, no. 9, pp. 997–1026, Jan. 2007.
[2] N. Masiera, A. Bojarska, I. Gawryszewska, E. Sadowy, W. Hryniewicz, and J. Waluk, “Antimicrobial photodynamic therapy by means of porphycene photosensitizers,” *J. Photochem. Photobiol. B Biol.*, vol. 174, no. July, pp. 84–89, 2017.



> P070. Poster

Symposium PDT-4 Human antimicrobial PDT (Tim Maisch)

PORPHYCENE TARGETING IN PHOTODYNAMIC THERAPY APPLICATIONS

Authors: Cormac Hally[12], Mireia Jordà-Redondo[1], Íngrid Nieves[1], Cristiano Viappiani[2], Montserrat Agut[1], Santi Nonell[1]
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Porphycenes are structural isomers of porphyrins that are promising photosensitising candidates thanks to their high $^1O_2$ photoproduction efficiency and their broad and strong absorption in the red/near-IR spectral region. Despite these desirable optical properties, their aggregation in water hampers their photophysical properties, and therefore reduces the effectivity of the therapy. The conjugation of porphycenes to hydrophilic targeting entities may enhance their solubility and would improve the selectivity of the treatment.

Herein, we present the conjugation of 2,7,12,17-methoxyethylporphycene to two hydrophilic entities, gentamicin as an antibiotic and triphenylphosphonium as a lipophilic cation. The synthesis and photophysical characterization of the compounds are reported, as also the biological *in vitro* assays of the gentamicin-porphycene conjugate on bacteria, fungi and mammalian cells. The conjugate presented interesting photophysical properties such as large singlet oxygen quantum yields, higher absorption in the red and tautomerism between to different photoactive species. Gentamicin-porphycene conjugate proved to inactivate mammalian cells, Gram-negative and Gram-positive bacteria in the submicromolar range, whilst higher concentrations were required for *C. albicans*.

Acknowledgements

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References

Planas, O. et al., Acid- and Hydrogen-Bonding-Induced Switching between 22-π and 18-π Electron Conjugations in 2-Aminothiazolo[4,5-c] Porphycenes. *Phys. Chem. Chem. Phys.* **2017**, *19* (37), 25537–25543.

Vogel, E. et al., Porphycene - a Novel Porphin Isomer. *Angew. Chemie Int. Ed.* **1986**, *25* (3), 257–259.

Cahan, R. et al., Photosensitizer-Antibiotic Conjugates: A Novel Class of Antibacterial Molecules. *Photochem. Photobiol.* **2010**, *86* (2), 418–425.

Murphy, M. et al., Targeting Antioxidants to Mitochondria by Conjugation to Lipophilic Cations. *Annu. Rev. Pharmacol. Toxicol.* **2007**, *47* (1), 629–656.

> 109. Invited Lecture

Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

USING LIGNIN FOR ANTIMICROBIAL PHOTODYNAMIC TREATMENT

Authors: Stéphanie Leroy-Lhez[1], Guillaume Marchand[1], Nicolas Villandier[1], Claude A. Calliste[1], Nidia Maldonado-Carmona[1,2], Tan S. Ouk[1], Mario J.F. Calvete[2], Mariette M. Peirera[2]
Presenting Author: Stéphanie Leroy-Lhez
1) University of Limoges, Laboratory PEIRENE 2) University of Coimbra, Department of Chemistry

Introduction

This work is a part of the H2020 EJD project "POLYTHEA: How light can save lives".1 One main issue of the POLYTHEA project is to develop photosensitizing dyes which can be used in a modular fashion for a variety of biomedical applications within the consortium.

Results and discussion

In this framework, nanoparticles of acetylated lignin could be considered as a biosourced vehicule for non-hydro soluble photosensitizers (PS) in Antimicrobial PhotoDynamic Treatments both for human health or agronomic applications. Their efficiency to produce reactive oxygen species (ROS) have been also evaluated thanks to Electron Spin Resonance.[2]

Conclusions

According to our work, acetylated lignins can be used as a potential photosensitizer, which opens the scope of their use in many areas such as, for instance, the eradication of harmful microorganisms. It represents a breakthrough in photodynamic treatment domains. Indeed, as it could be used alone in organic media or, thanks to its capacity to form nanoparticles in aqueous ones. Moreover, as for lignin-based nanospheres these nanoparticles should be able to encapsulate active compounds (Figure 1). With the objective to develop this methodology to non-hydro soluble porphyrins or phthalocyanine, first results will be presented on different biological systems.

Acknoledgements

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References

1. www.polythea.eu.
2. Marchand, G. *et al.; ChemistrySelect* **2018**, *3*, 5512–5516

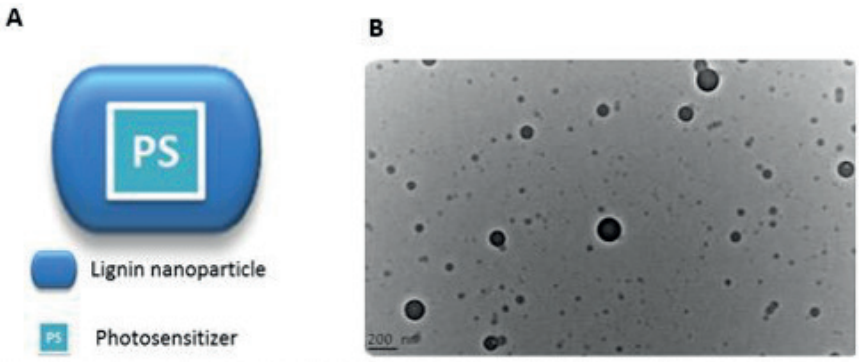

Figure 1 : A- Association between PS and nanoparticles; **B**- TEM picture of acetylated lignins nanoparticles encapsulating THPP.

> IL110. Invited Lecture
Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

AN INSIGHT INTO THE POTENTIATION EFFECT OF POTASSIUM IODIDE ON ANTIMICROBIAL PDT EFFICACY

Authors: Cátia Vieira[1], Adriele Santos[1,2], Ana T.P.C. Gomes[1], Mariana Q. Mesquita[3], Nuno M.M. Moura[3], M. Graça P.M.S. Neves[3], Adelaide Almeida[1], M. Amparo F. Faustino[3]
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Antimicrobial photodynamic therapy (aPDT) is gaining a special importance as an effective approach against multidrug-resistant strains responsible of fatal infections. In the literature is found that the addition of potassium iodide (KI) can increase the aPDT efficiency of some photosensitizers (PSs) on a broad-spectrum of microorganisms. In this communication we will discuss the KI effect on the aPDT of a broad range of positively charged porphyrins and non-porphyrinic PSs (*e.g.* Rose Bengal, and Toluidine Blue O, crystal violet or malachite green) in order to gain a more comprehensive knowledge about the potentiation by the coadjutant. The assays were performed using a bioluminescent *E. coli* strain as a model and the results indicate that the KI ability to potentiate the aPDT process is PS structure dependent.

For the PSs tested, the ones capable to decompose the peroxyiodide into iodine (easily detectable by spectroscopy or by the visual appearance of a blue color in the presence of amylose) were the most promising ones to be used in combination with KI. Although these studies confirmed that the generation of $^1O_2$ is an important fact in this process, the PS structure (charge number and charge position), aggregation behavior and affinity for the cell membrane are also important features to be taken in account.

Acknowledgements
Thanks are due to the University of Aveiro and FCT/MEC for the financial support to QOPNA (FCT UID/QUI/00062/2019), CESAM (UID/AMB/50017/2019) research units and the FCT project (FCT-PTDC/ASP-PES/29576/2017), to FCT/MEC through national funds, and the co-funding by the FEDER-Operational Thematic Program for Competitiveness and Internationalization-COMPETE 2020, within the PT2020 Partnership Agreement. Thanks are also due to the Portuguese NMR and Mass Networks. MM and NM thank to the Fundação para a Ciência e a Tecnologia FCT for their doctoral (SFRH/BD/112517/2015) and post-doctoral grants (SFRH/BPD/84216/2012), respectively.

References

Vieira C, Gomes ATPC, Mesquita MQ, Moura NMM, Neves MGPMS, Faustino MAF and Almeida A (2018). Front. Microbiol. 9:2665. doi: 10.3389/fmicb.2018.02665

Vieira C, Santos A, Mesquita MQ, Gomes ATPC, Neves MGPMS, Faustino MAF and Almeida A (2019*) J. Porphyrins Phthalocyanines,* in press, doi: 10.1142/S1088424619500408

> IL111. Invited Lecture
Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

AN EFFICIENT FORMULATION BASED ON CATIONIC PORPHYRINS TO PHOTOINACTIVATE GRAM POSITIVE AND GRAM NEGATIVE BACTERIA: IN VITRO AND EX VIVO EVALUATION.

Authors: Adelaide Almeida[1], Tatiana Branco[1], Vânia Jesus[1], Diana Martins[1], Márcia Braz[1], Maria Bartolomeu[1], Lúcia Marciel[1], Ana Gomes[1], Mariana Mesquita[2], Cristina Dias[2], Nuno Moura[2], Graça Neves[2], Amparo Faustino[2]
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Antibiotic resistance is a serious and growing worldwide problem with infections caused by multidrug-resistant microorganisms no more simply treated by antibiotics. Alternative tools are required to overtake this problem and antimicrobial photodynamic therapy (aPDT) seems a promising approach. Porphyrin derivatives have shown to be very promising sensitizers to aPDT but in many cases their synthesis requires time consuming and laborious processes related with chromatographic purifications.

In this study, it was evaluated the suitability of a formulation (**Form**) constituted by a non-separated mixture of porphyrins bearing different charges Mono-Py(+)-Me (19%), Di-Py(+)-Me *opp* and Di-Py(+)-Me *adj* (20%), Tri-Py(+)-Me (44%) and Tetra-Py(+)-Me (17%), obtained during the synthesis of the highly efficient photosensitizer Tri-Py(+)-Me. The effect of the **Form** to inactivate Gram positive and Gram negative bacteria was tested first in phosphate buffer solution (PBS) (*in vitro*) and after in different settings, relevant to clinical and environmental areas: pork skin, blood, kiwi leaves and residual water *(ex vivo*).

The results show that the **Form** was equally effective in the photoinactivation of Gram positive and Gram negative bacteria *in vitro* and *ex vivo* as the efficient Tri-Py(+)-Me and/or the well-known and recognized PS Tetra-Py(+)-Me used separately. The effective reduction of bacteria with the **Form** provided promising indications towards its use, which lead to a substantial decrease on costs and production time.

> IL112. Invited Lecture
Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

PHOTODYNAMIC MATERIALS FOR INFECTION PREVENTION IN HOSPITAL ENVIRONMENTS

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Introduction
Efforts to control hospital acquired infections have been hampered by the emergence of drug-resistant pathogens, necessitating the pursuit of self-disinfecting materials that are capable of eradicating such microbes in hospital environments. To that end, we have explored the feasibility of antimicrobial photodynamic inactivation (aPDI) of bacteria and viruses using photodynamic materials.

Methods
In vitro aPDI studies employing photosensitizer-embedded or conjugated cellulose nanocrystals, cellulose fibers, polyacrylonitrile nanofibers, or olefinic block copolymers were performed against bacteria and viruses. Pathogens were cultured, deposited onto the materials, and subsequently illuminated with visible light (400–700 nm, 65-80 mW/cm^2, 5-60 min), and their survivability was determined via colony counting or plaque assay methods.

Results and Discussion
For natural polymer scaffolds, cellulose-porphyrin conjugates (either as nanocrystals, nanofibers, or paper sheets) were found to be highly effective against a broad spectrum of pathogens: our best results demonstrated that *S. aureus*, *A. baumannii*, *P. aeruginosa* and *K. pneumoniae* all exhibited photodynamic inactivation by 99.99+%, as well as inactivation of dengue-1 virus (>99.995%), influenza A (~99.5%), and human adenovirus-5 (~99%).[1] As an alternative strategy, non-covalent approaches to photodynamic materials using artificial polymers were also explored: i) using electrospinning, cationic porphyrin and BODIPY photosensitizers were embedded into polyacrylonitrile and nylon nanofibers, and the resultant nonwoven materials possessed both antibacterial and antiviral activities;[2] ii) using melt-pressing, we developed a photosensitizer-embedded olefinic block copolymer that exhibited excellent antimicrobial properties against a range of microbes, including Gram-positive and Gram-negative drug-resistant bacteria, as well as against enveloped and non-enveloped viruses.[3]

Conclusions
Photodynamic materials may have widespread applicability for non-specific pathogen disinfection, and further research may lead to their application in hospitals and healthcare-related industries where novel materials with the capability of reducing the rates of transmission of a wide range of bacteria, viruses, and fungi, particularly of antibiotic resistant strains, are desired.

Acknowledgements
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Conflicts of Interest
The authors have no conflicts of interest to report.

References

1. Carpenter et al. “Synthesis, Characterization, and Antimicrobial Efficacy of Photomicrobicidal Cellulose Paper” *Biomacromolecules* **2015**, *16*, 2482.
2. Stanley et al. “Photosensitizer-Embedded Polyacrylonitrile Nanofibers as an Antimicrobial Non-Woven Textile” *Nanomaterials* **2016**, *6*, 77.
3. Peddinti et al. “Photodynamic Polymers as Comprehensive Anti-Infective Materials: Staying Ahead of a Growing Global Threat” *ACS Appl. Mater. Interfaces* **2018**, *10*, 25955.

> IL113. Invited Lecture
Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

DELIVERING PHOTOSENSITIZERS WITH PROTEINS: A BIOCOMPATIBLE AND LOW-COST APPROACH FOR PHOTOSENSITIZATION-BASED FOOD DECONTAMINATION

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Routine use of photosensitization for bacterial decontamination in food processing requires effective photosensitizing systems that are biologically compatible and available in large amounts at limited cost. New strategies for the realization of such systems are proposed, based on the combination of simple naturally-occurring components, like the photosensitizer (PS) Hypericin, extracted from flowers of *Hypericum perforatum*, and common endogenous proteins. In aqueous environment, Hypericin spontaneously binds to carrier proteins that are naturally abundant in non-processed food, like β-lactoglobulin in cow's milk or albumins and apo-myoglobin in meat. Spectroscopic methods demonstrate that stable complexes are formed in which the PS preserves its photo-activated properties, such as fluorescence emission and generation of reactive oxygen species, resulting in efficient inactivation of Gram-positive bacteria. Moreover, protein-based delivery of the PS to bacterial cells can be monitored directly by fluorescence imaging. Finally, the raw extract of *Hypericum perforatum* flowers, containing non-purified Hypericin, is used as a photosensitizing component. The spectroscopic properties of the PS and its interaction with albumin carriers allow a selective photo-activation of the Hypericin present in the mixture. Photo-inactivation of bacterial contaminants is achieved at considerably reduced costs. In conclusion, inexpensive photo-active systems are obtained, with great potential for treatment of Gram-positive bacteria contamination and fully compatible with food processing environments, without introduction of additional exogenous agents.

> IL114. Invited Lecture
Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

CHLOROPHYLLIN-BASED PHOTOSENSITIZATION AS INNOVATIVE NONTHERMAL APPROACH TO ENHANCE MICROBIAL SAFETY OF FRESH PRODUCE

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Despite tremendous progress in biomedical science, the number of reported food-borne diseases continues to rise. Health experts estimate that every year food-borne illnesses in USA cost 86 billion dollars. Obviously, existing antibacterial technologies for microbial control of foods are not enough effective.

Photosensitization is a treatment involving the interaction of the two non-toxic factors, photosensitizer and light, which in the presence of oxygen results in the destruction of the target cell without leakage of harmful by-products in the environment.

According to our results, chlorophyllin (food additive E140) exhibits perfect photosensitizing properties. After excitation with light (405 nm) it inactivates food pathogens, their spores and biofilms, yeasts/microfungi. ROS-induced oxidative stress and following membrane damage was the main reason of photosensitization- based inactivation of microorganisms.

Afterwards we applied photosensitization for microbial control of fresh produce. Obtained results indicate that this treatment significantly (2-3 log CFU/g) reduces microbial load on fruits (strawberries, apricots, plums), vegetables (cauliflower, cucumber, lettuce, basil) and sprouts without thermal effects on food matrix. Moreover, this treatment extended the shelf-life of treated produce by 2-4 days what is economically very important. No reduction of nutritional value (antioxidant activity, chlorophyll content) or organoleptic properties (color, texture, taste) of treated produce has been observed.

In order to decontaminate fresh produce from Gram (+) and Gram (-) pathogens in uniform way, chlorophyllin was conjugated with chitosan. Obtained data reveal that such photoactivated conjugate is very effective against all pathogens and can be applied for coating of fresh produce.

Therefore, a photosensitization phenomenon might open a new avenue for the development of non-thermal, effective and ecologically friendly antimicrobial technology for preservation of fresh produce.

> IL115. Invited Lecture
Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

PHOTODYNAMIC CONTROL OF AEDES AEGYPTI LARVAE BY USING EOSIN METHYLENE BLUE AS PHOTOSENSITIZER

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Aedes aegypti (*Ae. aegypti*) is a competent vector for transmitting important viral diseases such as yellow fever, dengue, chikungunya, and zika. Several strategies have been applied to avoid *Ae. aegypti* proliferation by using environmental management, biological, and chemical approaches. However, the development of new methods for effective control of the insect vector population is still needed. Photodynamic control is an alternative way to control the vector population by using a physical approach based on the larval phototoxicity of a photosensitizer. In this context, the present study evaluated the use of eosin-methylene blue (EMB) as a new photosensitizer for photodynamic control of *Ae. aegypti* larval populations. The photodynamic assays were performed submitting *Ae. Aegypti* third-instar larvae to different EMB concentrations (0.0, and 100.0 mgL^{-1}) in combination of three different light doses (96, 3 and 165, 06 J cm^{-2}) under either white-light radiation from RGB LEDs or wavelengths were used (450, 525 or 625nm). The results demonstrated that EMB presented a rapid internalization into the larvae. It has observed that the EAM is phototoxic for *Ae. Aegypti*. The photodynamic action is effective to control the larval populations of *Ae. Aegypti.* The larvae died using EAM and exposure to white light, on the different radiation wavelengths (450, 525 or 625nm).

> IL116. Invited Lecture

Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

SAVE THE CROP: PHOTODYNAMIC INACTIVATION BASED ON CHLORIN E6 DERIVATIVES AGAINST PHYTOPATHOGENIC FUNGI AND BACTERIA

Authors: Christoph Hamminger[1], Michael Glueck[1], Markus Hoerl[1], Jun Liu[2], Michael Fefer[2], Kristjan Plaetzer[1]
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Objective

The supply of the growing world population with sufficient and healthy food requires high plant densities, which promotes the spread of plant diseases. The overuse of pesticides to combat phytopathogens can lead to drug resistance and accumulation of unwanted substances in the environment. Photodynamic Inactivation (PDI) is a powerful approach to kill microorganisms [1]. This study investigates the efficacy of PDI based on two chlorin e6 derivatives, Sodium Magnesium Chlorophyllin (Chl, approved as food additive E140) and a water-soluble cationic amine substituted chlorin e6 (B170024) against bacterial and fungal plant pathogens.

Methods

PDI against the Gram(+) phytopathogen *Rhodococcus fascians* and Gram(-) *Erwinia amylovora* and *Xanthomonas axonopodis* was performed in liquid culture and illumination with 395 nm LED light (26.6 J/cm^2) or natural sunlight. CFU counting was done 24/48 h after phototreatment. For the antifungal PDI against *Alternaria solani* and *Botrytis cinerea* the growth of mycelial patches after photoactivation (395 nm, 106.6 J/cm^2) of either Chl or B17-0024 was measured for 7 days. Gram(-) and fungal phytopathogens were co-incubated with a cell wall permeabilizing agent. To investigate the effect of the PDI-treatment to host plants the growth of *Fragaria vesca* (BBCH stage 4) inoculated with the photosensitizers with or without additives was monitored for 14 days.

Results

Both photosensitizers photoinactivate *R. fascians*, with B17-0024 being effective (inactivation >6 $\log_{10}$ steps) at 10 µM and Chl at 100 µM. Photokilling (>7 $\log_{10}$) of Gram(-) bacteria with 100 µM Chl requires addition of Na_2EDTA (*X. axonopodis*) or Baypure®DS100 (*E. amylovora*). The cationic B17-0024 is phototoxic against both Gram(-) species at 10-100 µM without additives. Sunlight activation induces an antibacterial effect towards *R. fascians* after one hour (100 µM Chl, average light intensity 38 mW/cm²) and *E. amylovora* after two hours (100 µM B17-0024, 28 mW/cm²). Both chlorin e6 derivatives also act as very effective photofungicides: Chl is phototoxic if applied with Na_2EDTA, B17-0024 without any additives. The PDI-treatment had no negative effects on the growth of *F. vesca* plants.

Conclusion

PDI based on chlorin e6 derivatives is powerful in killing bacterial and fungal phytopathogens. Due to its excellent biocompatibility Chlorophyllin can be used as relatively inexpensive and ecofriendly photoactive compound, but requires synergistic additives such as chelators to kill Gram(-) bacteria as well as fungi. The cationic B17-0024 is phototoxic against all model pathogens tested in this study. As no negative effects to the host plants have been observed so far, the photodynamic approach could add to the grower\'s toolbox to protect the crops from plant diseases.

Reference

1. Wainwright, M., et al., *Photoantimicrobials - are we afraid of the light?* The Lancet Infectious Diseases, 2016. **17**(2): p. e49-e55.

> P071. Poster

Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

UTILIZING A THREE-PART WORKFLOW TO ELUCIDATE LIGHT-DEPENDENT DEFENSE STRATEGIES OF FUNGI

Authors: Fabian Hammerle[Unive], Pamela Vrabl[Unive], Isabella Bingger[Manag], Harald Schöbel[Manag], Hermann Stuppner[Unive], Bianka Siewert[Unive]
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Plants, which are not able to actively flee from threats have evolved a winning defense strategy based on photochemistry (Roberts & Paul 2006). In detail, light-activated defense strategies are based on the ability of certain pigments or so-called photosensitizers, to produce reactive oxygen species (e.g. $^1O_2$) by absorption of light (Flors & Nonell 2006). This led to our hypothesis that fungi – the second kingdom with "immobile" reproducing structures – might also possess highly active photosensitizers and thus the ability to utilize sunlight in order to fend off potential predators. To test our hypothesis, we established a workflow, which allows ranking fungal extracts according to their potential PDT-activity.

Methods

Dried fruiting bodies of several basidiomycetes were extracted with solvents of different polarity. Subsequently the extracts were submitted to a three-part screening-workflow (Siewert et al. 2019)consisting of HPLC-DAD-MS analysis, 9,10-dimethylanthracene (DMA)-assay and photocytotoxicity evaluation.

Results and Discussion

HPLC-DAD-MS analysis allowed us to group the extracts according to their pigment pattern. By employing the DMA-assay we were able to indirectly quantify the extracts´ ability to produce $^1O_2$ after irradiation with light. To validate the results of the DMA-assay *in vitro*, all extracts' light-dependent cytotoxicity was tested against cells from the cancer cell lines HeLa and A549.

While fungi containing pigments from the shikimate-chorismate pathway or the mevalonate pathway showed no significant activity, basidiomycetes containing dyes from the acetate-malonate pathways and nitrogen heterocycles were characterized by promising $^1O_2$-producing activities. Nevertheless, the conducted photocytotoxicity study showed that not all $^1O_2$-producing pigments are able to induce a photo-activated cytotoxic effect*in vitro*.

Conclusions

A photopharmaceutical workflow was established and validated with a well-known natural photosensitizer. This three-part workflow was utilized to test the hypothesis of a photochemical defense mechanism in the kingdom Fungi. By investigating a set of diverse basidiomycetes, we were able to show that colorants of the acetate-malonate pathway are promising photosensitizers.

Acknowledgment

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References

Flors C, Nonell S. Light and Singlet Oxygen in Plant Defense Against Pathogens: Phototoxic Phenalenone Phytoalexins †. *Acc Chem Res 2006;*39(5): 293–300

Roberts MR, Paul ND. Seduced by the dark side: integrating molecular and ecological perspectives on the influence of light on plant defence against pests and pathogens. *New Phytol 2006*; 170(4): 677–699

Siewert B, Pamela V, Hammerle F, Bingger I, Stuppner H. A convenient workflow to spot photosensitizers revealed photo-activity in basidiomycetes. *RSC Adv 2019;*9(8): 4545–4552

> P072. Poster

Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

PHYTOCHEMISTRY AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL OF MENTHA SUAVEOLENS EHRH

Authors: Hannou Zerkani[Resea], Smail Amalich[Resea], Touriya Zair[Resea]
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1) Research team of Bioactive MoleculesChemistry and Environment

During an ethnopharmacological survey carried out in the Khénifra region, we have noted that the most widespread and most treated pathologies are the digestive disorders. *Mentha Suaveolens* Ehrh is one of the herbs used to relieve these infections. Indeed, this study aims to confirm the traditional know-how of the population surveyed through the evaluation of the antibacterial activity of the essential oil, the identification of the chemical composition and the isolation of the active ingredients responsible for this activity by chromatographic and spectroscopic methods (CCM,CG, SM, RMN ^{1}H, RMN ^{13}C et RMN-DEPT ^{13}C).

Essential oil extracted from the aerial part of *Mentha Suaveolens* Ehrh, is obtained by hydrodistillation in a Clevenger type apparatus, its yield is 4.32% relative to the dry matter.

We selected nine microorganisms responsible for digestive infections to achieve the antibacterial activity of the essential oil of *Mentha Suaveolens* Ehrh, il is *Klebsiella pneumonia1, Escherichia coli (Résistante et Sensible), Staphylococcus aureus BLACT, Enterococcus faecalis, serratia fonticola,Acinétobacter baumannii,klebsiella oxytoca* and *Enterobacter aerogenes, P.aeuroginosa.* The essential oil of mentha suaveolens marked a strong activity with respect to *Klebsiella pneumoniae, Escherichia coli(resistance* and *sensible),Enterococcus faecalis, serratia fonticola, Acinétobacter baumannii* and *klebsiella oxytoca, however, it is inactive against Staphylococcus aureus BLACT, Enterobacter aerogenes* and *P.aeuroginosa.*

The essential oil of *Mentha Suaveolens* Ehrh showed a very strong antibacterial power compared to the standard antibiotics Fox 30, TIM 58 and PRL 100.

The analysis performed by GC / MS has allowed us to identify the chemical composition of the essential oil extracted from *Mentha Suaveolens* Ehrh, the major components of this HE are Piperitenone oxide (75.50%), piperitenone (5.55%), beta-caryophyllene (2.02%), limonene (1.68%), terpinen-4 -ol (1.27%) and pulegone (1.05%).

The antibacterial activity of the marked *Mentha Suaveolens* Ehrh essential oil is related to its chemical composition, Indeed, this species was fractionated on an open column of silica, using an eluent (hexane / ether), of increasing polarity with a view to isolating Piperitenone oxide (75.50%) and piperitenone (5.55%).

> P073. Poster

Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

PHOTODYNAMIC INACTIVATION AGAINST PHYTOPATHOGENIC FUNGI

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Objective

The growing world population renders global nutrition with healthy and safe food one of the major challenges of years to come. The global crop loss caused by bacterial and fungal pathogens is estimated around 16% [1]. The standard treatment to combat phytopathogens in industrialized monocultural farming is by using conventional pesticides. These harsh chemicals may leach into soil and water. Photodynamic Inactivation (PDI) has proven to be a powerful approach to kill microorganisms [2]. We here investigate the efficiency of PDI based on B17-0024, a cationic amine substituted chlorin e6, or a formulation of Sodium Magnesium Chlorophyllin (Chl) with Na_2EDTA as cell wall permeabilizing agent against two relevant phytopathogenic fungal model organisms.

Methods

For the antifungal PDI of *Alternaria solani* and *Botrytis cinerea* mycelia were grown in liquid medium for 24 hours (*A. solani*) or 48 h (*B. cinerea*). Small spheres of the mycelia (average diameter 2 mm) were incubated for 100 min with 1 µM, 10 µM or 100 µM B17-0024 or Chl with and without 5 mM Na_2EDTA. Samples were illuminated with 395 nm (radiant exposure 106.6 J cm^{-2}) and the radial growth of mycelial patches after 7 days on agar medium was measured. Fungal mycelia were considered dead, if no growth was observed.

Results

B17-0024 showed high phototoxicity against both fungal model organisms. Growth of mycelial patches of *A. solani* was completely inhibited after PDI with 10 µM B17-0024. For *B. cinerea* a 10-fold higher concentration (100 µM) was required to achieve 100 % photokilling. Using Chl alone as photosensitizer did not result in a fungicidal effect, but upon addition of 5 mM Na_2EDTA the treatment resulted in an inactivation of mycelial patches of 94.1 % for *A. solani* and 91.7 % for *B. cinerea*.

Conclusion

The cationic and water-soluble B17-0024 completely kills both fungal phytopathogens employed in this study at considerably low concentrations and without additional additives. If combined with a cell wall permeabilizer, the cost-efficient and readily available Chl (approved as food additive E140) can act as effective photofungicide. Thus, PDI based on Chl and its derivative B17-0024 could add to the grower's toolbox to overcome crop losses caused by phytopathogens.

References

1. Oerke, E.C., *Crop losses to pests.* The Journal of Agricultural Science, 2005. **144**(1): p. 31-43.
2. Wainwright, M., et al., *Photoantimicrobials - are we afraid of the light?* The Lancet Infectious Diseases, 2016. **17**(2): p. e49-e55.

> P074. Poster

Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

PHOTODYNAMIC INACTIVATION OF BACTERIAL PLANT PATHOGENS BASED ON THE CATIONIC PORPHYRIN B17-0024

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Objective

Bacterial plant pathogens pose an economic threat to growers, especially if the infections are out of reach of conventional treatment modalities. Photodynamic Inactivation represents a novel and effective antimicrobial approach, which could add to the farmers toolbox to fight plant diseases. We here determine the efficiency of a cationic, water-soluble chlorin e6 derivative tailored for the application on plants, B17-0024, in photokilling of three relevant bacterial phytopathogens: i) Gram(+) *Rhodococcus fascians* which leads to leafy galls on ornamentals and strawberries ii) Gram(-) *Erwinia amylovora*, the cause of fire blight in *Rosaceae* such as apples and pears, and iii) Gram(-) *Xanthomonas axonopodis* (its pathovar *citri* causes citrus canker). *X. axonopodis* and *E. amylovora* both rank on the top ten bacterial plant pathogens list published by Mansfield et al in 2012[1].

Methods

The bacteria were cultivated at 26 °C in a shaking incubator and suspensions were incubated with either 1, 10, 50, 100 or 200 µM B17-0024 for 5 and 30 minutes in the dark under constant agitation. Illumination was performed using a LED array (395 nm, max. 26.6 J/cm^2 radiant exposure). Results were evaluated by determination of CFU (after two days for *E. amylovora* and five days for *R. fascians* and *X. axonopodis*) and calculation of the relative inactivation.

Results

Against Gram(+) *R. fascians*, PDI based on B17-0024 was able to unleash its bactericidal potential at 10 µM (inactivation > 6 log steps at 5 and 30 minutes incubation period). *X. axonopodis* can be successfully photosanitized by B17-0024 at concentrations above 10 µM (inactivation of up to 7 log steps for 5 and 30 min incubation). B17-0024 successfully reduced the number of viable *E. amylovora* by up to six log steps, using a concentration of 100 µM and an incubation period of 30 minutes - and a 4 log reduction at five minutes incubation. B17-0024 shows no dark toxicity against the tested bacterial strains at the tested concentrations.

Discussion

PDI based on the cationic chlorin e6 derivate B17-0024 is effective in photokilling of both Gram(+) and Gram(-) bacterial phytopathogens - thus making it a true broad-spectrum photoantimicrobial agent which does not require additional cell wall permeabilizing additives. The excellent water-solubility of B17-0024 paves the way for application in aqueous solution without organic solvents. This photosensitizer (PS) could allow for economic field application due to its fairly low effective concentration. Since at 100 µM even 5 minutes of incubation are sufficient for an antibacterial effect it could be applied within normal farming practice.

References:

[1] J. Mansfield, S. Genin, S. Magori, V. Citovsky, M. Sriariyanum, P. Ronald, M. A. X. Dow, V. Verdier, S. V. Beer, M. A. Machado, I. A. N. Toth, G. Salmond, and G. D. Foster. Top 10 plant pathogenic bacteria in molecular plant pathology. Molecular Plant Pathology, 13(6):614–629, June 2012.

> P075. Poster

Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

ANTIBACTERIAL POLYSTYRENE NANOPARTICLES WITH TETRAPHENYLPORPHYRIN PHOTOSENSITIZER

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Nanomaterials releasing antibacterial agents (e.g. singlet oxygen, $^1O_2$) are garnering increasing interest, as they might fill the gaps where antibiotics frequently fail. Polystyrene (**PS**) nanoparticles with encapsulated tetraphenylporphyrin (TPP) photosensitizer (**TPP@PS)** were prepared by nanoprecipitation method from electrospun nanofiber material [1] and characterized by photophysical methods to predict their properties for selected biological applications and also to obtain additional information about location and properties of a dye inside nanostructures.

The photophysical processes after excitation of TPP include formation of the TPP triplet states (followed by transient absorption at 460 nm) and antibacterial $^1O_2$ (followed by luminescence at 1270 nm). We also evaluated singlet oxygen sensitized delayed fluorescence (SODF followed by luminescence at 640 nm)

TPP@PS can serve as container of $^1O_2$ with its gradual release to their environment. TPP is fixed in glassy structure of nanoparticles. Lifetime of singlet oxygen photogenerated by smaller **TPP@PS** (DLS size ~10-30 nm) decreased significantly from τ_Δ~20 µs typical for **PS** bulk due increased release of $^1O_2$ to the environment, where quickly deactivated (τ_Δ = 3.5 µs in H_2O). The kinetics of TPP triplets, $^1O_2$ and SODF were significantly influenced by the temperature due to changes in the oxygen diffusion and oxygen solubility, both in **PS** matrix and in their surrounding environment. A simple method based on SODF permitted the *in situ* continuous measurement of the dissolved oxygen concentration in aqueous media in the broad region of oxygen concentrations from anaerobic conditions to oxygen-saturated media without the addition of any external oxygen sensor. Finally, a strong antibacterial effect observed on *Escherichia coli* indicates that **TPP@PS** is a promising material for antibacterial applications triggered/modulated by light.

Conclusion

The formation of $^1O_2$, together with additional thermal effects and concurrent optical monitoring capabilities by analyzing SODF kinetics, are the effective characteristics of **TPP@PS** multifunctional nanoplatform, which is particularly promising for utilization in various fields where antibacterial properties are highly required.

Acknowledgement

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References

1. Kubát, P.; Henke, P.; Berzediová, V.; Stepanek, M.; Lang, K.; Mosinger, J., *ACS Appl. Mater. Interfaces 2017, **9**, 36229*.

> P076. Poster

Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

INTEGRATED PHOTOSENSITIZING ADSORBENT MATERIAL FOR THE REMOVAL OF TRICLOSAN FROM WATER

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Many of the chemicals present in everyday pharmaceuticals and personal care products end up in the aquatic environment through wastewater treatment plants, as currently available water treatment technologies are ineffective for the complete removal of these microcontaminants from water.[1] Therefore, the continuous input of these chemicals into the environment is a serious concern that has to be addressed.

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol, TCS) is an antimicrobial agent widely used for over 30 years in personal care products. Since its chemical structure resembles that of thyroid hormones, TCS might disrupt thyroid hormone homeostasis.[2] Moreover, this chemical might also contribute to the development and spread of antibacterial resistance, that could lead to infections not treatable with the existing clinical antimicrobial agents.[3] Once in the environment, TCS in its neutral form (pH ≤ 7.9) has high bioaccumulation potential and environmental persistence because of its lipophilicity, and TCS has shown toxic effects in algae, phytoplankton and other aquatic organisms.[4]

The use of integrated photocatalyst/adsorbent systems for the photodegradation of water contaminants is an appealing concept for achieving the complete removal of water micropollutants.[5] Singlet oxygen photosensitizers immobilized on porous hydrophobic polymers might be suitable integrated photosensitizer/adsorbent materials for the removal of water microcontaminants using solar reactors.[6] In particular, TCS is strongly adsorbed by porous silicone and the pseudo-first order rate constant for TCS removal in the presence of a photosensitizing material based on porous silicone shows 4.6 times faster kinetics than direct photolysis of TCS (1.3 times faster than TCS adsorption), while in the case of deprotonated TCS (where TCS^- adsorption is negligible) the kinetics still are 1.7 times faster in the presence of the photosensitizing material, *i.e.*, 350 mg/L TCS may be removed from water in approx. 1 h under sunlight, *vs*. 4 h for direct photolysis.

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There are no conflicts to declare.

References

[1] (a) A. Gogoi *et al*. *Groundw. Sustain. Dev*., **2018**, *6*, 169-180; (b) Z. Baalbaki *et al*. *Chemosphere*, **2017**, *178*, 439-448.
[2] M. F. Yueh and R.H. Tukey, *Annu. Rev. Pharmacol. Toxicol*., **2016**, *56*, 251-272.
[3] (a) F. I. Sonbol *et al. J. Appl. Microbiol*., **2019**, *126*, 730-739; (b) J. Lu *et al. Environ. Int*., **2018**, *118*, 257-265.
[4] (a) E. Gorenoglu *et al. J. Environ. Management*, **2018**, *211*, 218-224; (b) L. Chenguang *et al. Environ. Sci. Pollut. Res*., **2018**, *25*, 9636-9646.
[5] (a) D. Kanakaraju *et al. Appl. Catal. B, Environ*., **2015**, *166-167*, 45-55; (b) N. Yahya *et al. J. Environ. Chem. Eng*., **2018**, *6*, 7411-7425.
[6] (a) E. Díez-Mato *et al. Appl. Catal. B, Environ*., **2014**, *160-161*, 445-455; (b) D. García-Fresnadillo. *ChemPhotoChem*, **2018**, *2*, 512-534.

> P077. Poster

Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

CHITOSAN-PHOTOSENSITIZER CONJUGATES: SYNTHESIS, PHOTOPHYSICAL CHARACTERIZATION AND PHOTODYNAMIC ANTIFUNGAL ACTIVITY EVALUATION. DEVELOPING A FORMULATION FOR CROP PROTECTION

Authors: Luciano Dibona[1]
Presenting Author: Luciano Dibona
1) Pontificia Universidad Católica

Pathogenic Fungi presence in plants and fruits are an alarming problem and causes production losses in the agricultural industry reaching 50% of production losses in developing countries. The use of traditional fungicides partially solves this problem but leaves serious consequences such as harmful residues on fruits, environmental contamination and the apparition of resistant fungal strains.

A promising alternative to traditional fungicides is using Antimicrobial Photodynamic Therapy (aPDT) as a platform to develop brand new fungicidal formulations based on the cytotoxic effect of reactive oxygen species (ROS), specifically Singlet Oxygen ($^1O_2$) generated by a photosensitizer (PS) and the irradiation of light. The results reported in the literature indicate that aPDT effectively has fungicidal activity in different strains of fungi.

Another alternative to traditional fungicides is Chitosan (CH). This polymer is obtained by a deacetylation process of chitin obtained from crabs and shrimps shells is widely used as a fungicide in the agricultural industry due to its low price, biodegradability and non-toxic to the human consumption[1].

There is evidence in the literature that support the idea of using CH and PS together in aPDT. Examples are Rose Bengal and a Chitosan polycationic derivative covalent conjugate against *E. faecalis* and *P. aeruginosa* Bacteria[2] and Chlorophyllin-Chitosan physical blend against *L. monocytogenes* Bacteria[3], both research concludes that the addition of Chitosan to the formulation enhances in some extent the photodynamic cytotoxic activity of the photosensitizer.

Under this context, we propose using both aPDT and CH as a fungicide formulation through the synthesis of covalent conjugates between low molecular weight chitosan (CH_H) and two PS separately (PS_1 and PS_2). Specifically, Photophysics properties such as Fluorescence and Absorption Spectra, Time-Resolved Fluorescence and Anisotropy, Triplet Absorption were studied as well as Photochemical processes such as Singlet Oxygen generation. Results between PS and CH_H-PSs were compared. Finally, to confirm the photodynamic antifungal activity of the formulations, *in vitro* analysis was made against *Penicillium Digitatum* using PS, CH_H and CH_H-PS as fungicide agents through CFU counting and Fungal radial growth measurements among others.

References

1. A. El Hadrami, L. R. Adam, I. El Hadrami and F. Daayf, *Mar Drugs*, 2010, **8**, 968-987.
2. A. Shrestha and A. Kishen, *Photochem Photobiol*, 2012, **88**, 577-583.
3. Z. Luksiene and I. Buchovec, *International Journal of Food Processing Technology*, 2015, **2**, 26-30.

> P078. Poster

Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

MIND THE GAP: TOLERANCE OF GRAY MOLD FOR OPTICAL BASED MANAGEMENT IN RELATIONS TO POWDERY MILDEWS

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Based on the efficiency on inhibition of developmental stages of *Oidium neolycopersici*, the causal agent of powdery mildew in tomato, UV (peak wavelength at 254 nm) was tested for its effect on colony growth and sporulation of *Botrytis* isolates, the causal agents of gray mold in strawberry/tomato. *Botrytis* isolates of B05.10, T4 and field isolates of 96/16-14.1 and 96/16-9.2 (hereafter referred as 1 and 2, respectively) were selected and cultured in Petri dishes containing potato dextrose agar (PDA). Petri dishes were sealed and incubated in controlled environment chambers for five days with an air temperature of 22 ± 1°C and RH of 75 ± 5%. Daily light cycles of 12:12 h dark:light (D:L) was provided with high pressure mercury lamps with an irradiance of 124.7± 8.6 µmol/m^2/s. Non sporulating mycelial plugs (5 mm in diameter) were excised from the active growing edge and transferred to the center of new PDA plates (one plug per plate). Petri dishes were sealed with thin poly ethylene film that can transmit all UV and visible range. Petri dishes were incubated in controlled environment chambers with an optical environments of i) No UV, 12:12 h D:L, ii) 30 seconds of UV, 12:12 h D:L, and iii) 60 seconds of UV, 12:12 h D:L. The UV irradiance was 8±0.5 µmol/m^2/s. After three days of incubation, colony diameter was measured. Based on the results, a second experiment was conducted in similar fashion with 2 and 4 min. of UV instead of 30 and 60 seconds. Colony diameter was measured three days after incubation and sporulation behavior was examined 7 and 14 days after incubation. Exposure to UV for 30 or 60 seconds had no effect on colony diameter relative to control (no-UV). Increasing duration of UV exposure to 4 min had significant effect on the colony diameter ($P = 0.0001$). Relative to control treatment, *Botrytis* isolates of B05.10, T4 and field isolate 1 had significantly smaller colony diameter when they were exposed to 4 min. of UV. Compared with non-UV control, all UV treated isolates had sparse colonies. Under non-UV treatment, B05.10 and T4 showed profound sporulation (macroconidia) followed by field isolates 1 and 2. All of these isolates formed significantly less number of macroconidia with daily UV treatment for 1 min or more. Daily application of UV (with peak wavelength emission of 254 nm) during night significantly suppressed growth and sporulation of *Botrytis* species in a dose dependent manner. Longer duration of UV exposure is necessary to achieve significant suppression on *Botrytis* species compared with *O. neolycopersici* (30 seconds). While fungi causing powdery mildew lack UV screening mechanisms, the *Botrytis* species causing gray mold are melanin pigmented with active optical screening mechanisms. This may give them a higher UV tolerance than the fungi causing powdery mildew. At present, we are working on possible wavelength combinations within optical radiation range to enhance the efficiency of UV in suppression of *Botrytis* species.

> P079. Poster

Symposium PDT-5 Environmental aPDT (Kristjan Plaetzer)

ANTIMICROBIAL PHOTODYNAMIC THERAPY FOR CONTROL OF FARM FISHES PATHOGENS

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The increasing resistance of pathogenic microorganisms towards antibiotics reinforces the research and development of new strategies among which is the antimicrobial photodynamic therapy (aPDT). The farm fishes production offently suffers from high level of contamination of table fishes due to chemicals treatments. Moreover, the more efficient alternatives to control the crustaceans infections can have the solution in aPDT. The present study aims evaluation of the succesability of two resistant strains *Flavobacterium psychrophilum* and *Aeromonas hydrophila* and their sensivive species towards phthalocyanines within the PDT method. *F. psychrophilum* is a Gram-negative bacterium which is found in cold fresh waters with an optimal growth temperature below 16 °C. *A. hydrophila* is Gram-negative bacterium that cause zoonotic diseases (means they can spread from animals to humans and vice versa) and this strain can be isilated from the both animal and plant food products. The study was carried out with Zn(II) phthalocyanine with methylpyridyloxy-groups as peripheral substituents (ZnPcMe). Four experimental groups of bacterial suspensions of 10^6 CFU per mL, three were controls and one PDT treated with ZnPcMe (1.25 - 10 μM) and LED 635 nm (60 J/cm^2) were studied. The results showed lack of dark toxicity as well as of only light toxicity. The PDT treated groups of *F. psychrophilum* were fully inactivated, but at the same protocol conditions *A. hydrophila* showed 3 log efficiency of inactivation. The antibiotic-graphs are also tested and presented the resistancy of the both strains towards wide group of chemotherpeutics. The nest step of experimentals will answers the practical usage of the PDT approach. As a conclusion can be said that aPDT looks prospective methodology to keep under control the diseases in farm fishes

Acknowledgements

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> IL117. Invited Lecture
Symposium PDT-6 PDT and oxygen (Theresa Busch)

A ROLE FOR TUMOR PHYSIOLOGY IN PERSONALIZATION OF PHOTODYNAMIC THERAPY

Authors: Theresa Busch[1]
Presenting Author: Theresa Busch
1) University of Pennsylvania

Introduction

Photodynamic therapy (PDT) with conventional photosensitizers is an oxygen-dependent process, and correspondingly, the efficacy of PDT has been associated with tumor oxygenation before or during light delivery. Many factors collectively determine the oxygenation of a tumor. Intervascular distances and vascular perfusion are key determinants of access to oxygenated blood, while the phenomenon of photochemical oxygen consumption and PDT-induced vascular damage can dictate tumor oxygenation during light delivery.

Methods

We have studied the association between tumor oxygenation and treatment outcome to PDT in both the clinical and preclinical settings. Lesion oxygenation was measured before and after PDT in patients with early stage/premalignant disease of the head and neck. In animals, both tumor oxygenation and blood flow were studied in relation to PDT outcome. We developed an interactive platform for PDT light delivery that adjusts treatment fluence rate based on real-time monitoring of tumor blood flow. We have considered how this platform can be used to conserve tumor oxygenation during illumination with potential benefit to long-term outcome.

Results

In our clinical trial, patients experienced a more durable treatment outcome if their lesions of head and neck (pre) malignancy were more highly oxygenated[1]. In preclinical studies, we've previously shown PDT effect on both tumor oxygenation and blood flow to be predictive of treatment outcome[2-3]. We've now studied how oxygen-depleting high fluence rate PDT can be modulated during light delivery in an interactive process that is based on real-time measurement of tumor blood flow. Using this blood-flow informed technique, fluence rate is lowered during treatment for lengths of time that are sufficient to permit blood flow recovery. This iterative process resulted in significantly higher tumor oxygenation at the conclusion of PDT compared to high fluence rate treatment. This was accompanied by significant improvements in outcome.

Conclusions

Tumor oxygenation is a well-known determinant of outcome to PDT with many photosensitizers, as demonstrated in both preclinical studies and clinical trials. Interactive approaches to light delivery may provide a personalized means to conserve tumor oxygenation and provide for a better therapeutic effect.

References

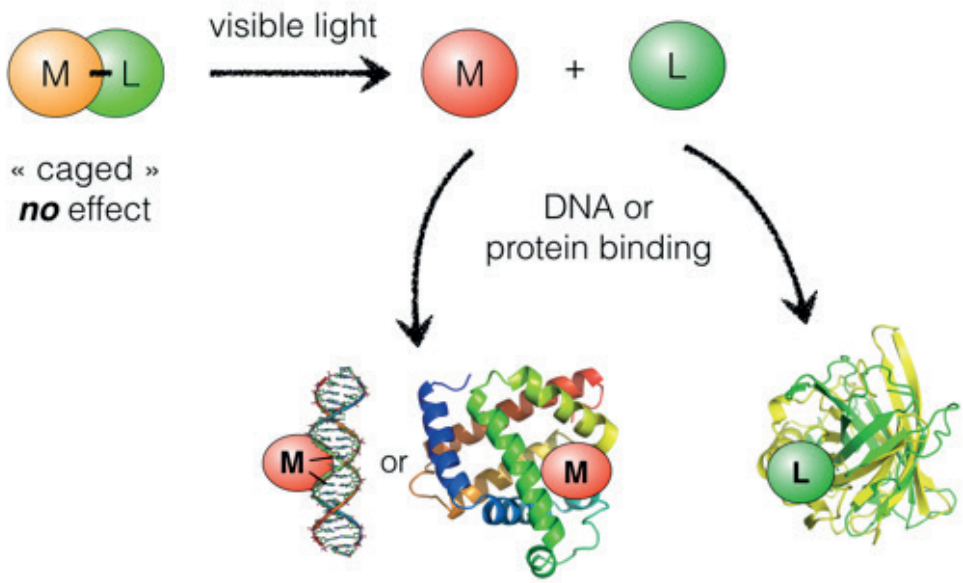
[1] Ahn PH, et al. *Lesion oxygenation associates with clinical outcomes in premalignant and early stage head and neck tumors treated on a phase 1 trial of photodynamic therapy*. Photodiagnosis Photodyn Ther. 2018 Mar;21:28-35. doi: 10.1016/j.pdpdt.2017.10.015. PMC5866751.
[2] Yu G, et al. *Noninvasive monitoring of murine tumor blood flow during and after photodynamic therapy provides early assessment of therapeutic efficacy*. Clin Cancer Res. 2005 May 1;11(9):3543-52. PubMed PMID: 15867258.
[3] Wang HW, et al. *Treatment-induced changes in tumor oxygenation predict photodynamic therapy outcome*. Cancer Res. 2004 Oct 15;64(20):7553-61. PubMed PMID: 15492282

> IL118. Invited Lecture
Symposium PDT-6 PDT and oxygen (Theresa Busch)

PHOTOACTIVATED CHEMOTHERAPY IN HYPOXIC CANCER CELLS

Authors: Sylvestre Bonnet[1]
Presenting Author: Sylvestre Bonnet
1) Leiden University

Photo-Activated Chemotherapy (PACT), like PhotoDynamic Therapy (PDT), aims at activating anticancer medicines with visible light to circumvent to the tumour site the toxicity of traditional chemotherapy. Unlike PDT, PACT agents are activated by the photocleavage of a metal-ligand bond. As this activation mechanism is independent from the presence of dioxygen in the irradiated tissues, it is also working in hypoxic conditions, where PDT often fails. In this presentation, several PACT compounds based on ruthenium will be presented that can be activated with blue, green, or red light. In some of them, it is the metal-based photoproduct that is responsible for the light-induced cytotoxicity, while in other cases it is the ligand that provokes cell death. In any case, we will provide the first experimental evidence that activation also works in hypoxic cancer cells.



> IL119. Invited Lecture
Symposium PDT-6 PDT and oxygen (Theresa Busch)

TRANSITION METAL COMPLEXES AS PHOTOSENSITZERS FOR PDT AND PCT

Authors: Sherri McFarland[1], Colin Cameron[1], Susan Monro[2], John Roque III[1], Liubov Lifshits[1], Patrick Barrett[1], Houston Cole[1]
Presenting Author: Sherri McFarland
1) The University of North Carolina at Greensboro 2) Acadia University

There has been an ongoing interest in the development of new photosensitizers (PSs) for photodynamic therapy (PDT), specifically to address some of the drawbacks associated with the pyrrole-based PSs that are most commonly employed. A related area of active investigation has been the design of PSs with novel mechanisms of action, including oxygen-independent photoprocesses (known as photochemotherapy, or PCT) or the capacity to switch to such modes at low oxygen tension. Since tumor hypoxia can present a real challenge for PDT in certain cases, PSs that operate through PCT mechanisms offer the possibility of treating some of the most aggressive and drug-resistant tumors that resist the traditional photodynamic reactions.

Transition metal complexes have emerged as attractive PSs for both PDT and PCT. Certain coordination complexes of ruthenium (Ru) are potent phototoxins toward a variety of in vitro and in vivo cancer models. One example is our own TLD1433, which is the first Ru PS to enter (and successfully complete) a human clinical trial for treating cancer with PDT. Part of the interest in Ru PSs stems from the ability to access a variety of excited state electronic configurations with visible or near-infrared light by judicious choice of ligand combinations around the metal center. These excited states, in turn, may participate in traditional type I/II photodynamic reactions as well as oxygen independent pathways that form the basis of PCT. In this conference presentation, we will discuss the design and development of transition metal complex PSs that exploit both PDT and PCT effects. The emphasis will be on the structural features and photophysical models that give rise to excited triplet states with characteristic reactivities.

> IL120. Invited Lecture
Symposium PDT-6 PDT and oxygen (Theresa Busch)

DIRECT IMAGING OF SINGLET OXYGEN LUMINESCENCE IN BLOOD VESSELS OF DORSAL SKINFOLD WINDOW CHAMBER MODEL

Authors: Buhong Li[1], Brian C. Wilson[2]
Presenting Author: Buhong Li
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Introduction
Singlet oxygen is a highly oxidative reactive oxygen species that plays an important role in numerous chemical and photochemical reactions. In particular, singlet oxygen is widely recognized as the key reactive oxygen species mediating the photodynamic effect via type-II of photosensitization, and this effect is the basic mechanisms of photodynamic therapy (PDT). Quantification of singlet oxygen generation during photosensitization are of immense importance of value for both preclinical research and future clinical practice.

Methods
A novel configuration of near-infrared (NIR) sensitive InGaAs camera has recently developed that enables directly image the singlet oxygen luminescence at 1270 nm generated in blood vessels in a dorsal skinfold window chamber model *in vivo* during vascular targeted PDT (V-PDT).

Results and Discussion
The NIR signals identification were performed successfully on the Rose Bengal solution environment and in a mouse skinfold window chamber *in vivo*, respectively. Furthermore, a total treatment fluence of 30 J/cm^2 of 532 nm light for 5.0, 15.0 and 25.0 mg/Kg BW three different RB dosages groups mice were implemented to test the correlation between the cumulative singlet oxygen luminescence and the vasoconstriction of specific regions of interest (ROIs) of blood vessel.

Conclusions
This study firstly demonstrated the capacity of the newly-developed sensitive system for imaging of singlet oxygen luminescence, and there was a strong correlation between the cumulative singlet oxygen luminescence and the vasoconstriction of blood vessel immediately after V-PDT treatment. This system has potential for establishing singlet oxygen luminescence based dosimetry for V-PDT.

Acknowledgements
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Conflicts of Interest
The authors declare there is no conflicts of interest regarding the submission of this abstract.

References

[1] Lin L, Lin H, Chen D, Xie S, Gu Y, Wilson BC, Li B. *SPIE Newsroom*. 2014, 10.1117/2.1201405.005511.
[2] Wilson BC, Patterson MS, Li B, Jarvi MT. *J Innov Opt Health Sci*. 2015, 8(1): 1540006.
[3] Li B, Lin L, Lin H, Wilson BC. *J Biophotonics*. 2016, 9(11-12):1314-25.
[4] Li B, Zhang J. Proceedings of ACP, 2016, AF1K.1.
[5] Pfitzner M, Schlothauer JC, Lin L, Li B, Röder B. *Imaging in Photodynamic Therapy*. 2017, CRC Press, 67-87.

> IL121. Invited Lecture
Symposium PDT-6 PDT and oxygen (Theresa Busch)

THE EFFECT OF SURGERY ON OUTCOMES IN INTRAOPERATIVE PDT: IS GREAT OXYGENATION AN EXTINCTION LEVEL EVENT FOR CANCER?

Authors: Keith Cengel[1]
Presenting Author: Keith Cengel
1) University of Pennsylvania

Patients with malignant pleural mesothelioma treated with lung sparing surgical resection and intraoperative photodynamic therapy (PDT) demonstrate remarkable overall survival outcomes. In our clinical and preclinical studies, we have shown that surgical debulking unequivocally contributes to successful PDT outcomes by reducing the number of cells that need to be treated. Nevertheless, local (pleural) recurrence remains a significant problem, suggesting that improved local PDT efficacy would be clinically beneficial. Here, we present our findings the effects of surgical debulking on critical determinates of PDT efficacy, including tumor oxygenation, photosensitizer uptake and host anti-tumor immune response. In both clinical and preclinical studies, we have found that intrinsic and PDT-induced heterogeneity in tumor oxygenation and photosensitizer uptake strongly affect PDT outcomes, but surgically induced changes in these parameters are unlikely to explain the potentially negative effects of surgery itself on PDT efficacy. Moreover, we found that surgically induced changes in innate and adaptive immunity can significantly impair PDT efficacy. Taken together, these data suggest that improving intrinsic and PDT-induced heterogeneity in tumor oxygenation and photosensitizer uptake while addressing the negative immunologic consequences of surgical resection will improve outcomes for intraoperative PDT.

> IL122. Invited Lecture
Symposium PDT-6 PDT and oxygen (Theresa Busch)

FLUENCE RATE EFFECTS IN INTERSTITIAL PHOTODYNAMIC THERAPY

Authors: Gal Shafirstein[1], David Bellnier[1], Emily Oakley[1], Michael Habitzruther[1], Sasheen Hamilton[1], Hannah Cooper[1], Alan Hutson[1], Sandra Sexton[1], Leslie Curtin[1], Joseph Spernyak[1], Steven Turowski[1], Hassan Arshad[1], Lawrence Tworek[1], Matthew Mallory[1], Barbara Henderson[1]
Presenting Author: Gal Shafirstein
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Introduction
Because light penetration in tissue is limited, interstitial PDT (I-PDT) is required to treat locally advanced cancers (LAC). In I-PDT, multiple cylindrically diffusing optical fibers (CDF) are placed in the tumor. Recently we reported [1] that effective I-PDT of large animal tumors requires threshold fluences and fluence rates within the tumor and margins. In this talk I will describe our preclinical study and discuss the role of fluence rate in clinical I-PDT.

Methods
C3H mice bearing large subcutaneous SCCVII tumors (400-600 mm^3), and New Zealand White (NZW) rabbits bearing intramuscular VX2 carcinoma tumors (3200-6400 mm^3), were injected intravascularly with porfimer sodium (Photofrin®) 24 hours before light treatment. Light at 630 nm from diode lasers was delivered through CDFs implanted in the tumors. Image-based finite element method was employed to compute the fluence rates within 100% of the volume of the tumors and margins. The intratumoral fluence rate was monitored with light dosimetry system. Magnetic resonance thermometry (MRT) was used to study the intratumoral temperature in mice. Tumor responses were assessed with caliper measurements and computed tomography (CT) in mice and rabbits, respectively.

Results and Discussion
A significantly ($p < 0.05$) higher cure rate (70-90%) was observed in mice treated with I-PDT relative to light only at intratumoral fluence rates of 8.4–245 $mW/cm^2$and fluence of ≥45 J/cm^2[1]. A maximum of 25% cure was observed when the fluence rate was <8.4 mW/cm^2. Increases in toxicity were observed when the maximum intratumoral fluence rate was ≥245 mW/cm^2. Nonuniform temperature distributions were observed with MRT and that was attributed to light absorption by blood [1]. In rabbits, I-PDT with 16.5–398 mW/cm^2 and ≥45 J/cm^2 resulted in local control. Although light alone induced tissue heating in the VX2 tumors, the thermal effects did not result in local control; this was likely due to the larger VX2 tumor volume compared to the SCCVII. Our studies show that I-PDT differs markedly from external beam PDT in terms of light dosimetry and the generation of photothermal effects.

Conclusions
In I-PDT, a range of high fluence rate thresholds is required to achieve effective control of LAC.

Conflicts of Interest
G.S, D.B, and E.O are co-inventors in RPCI patent applications. G.S and H.A acknowledge research grant support from Concordia Lab. Inc. G.S. acknowledges a service on the advisory board for Concordia Int. Corp. and Pinnacle Biologics, Inc. All other co-authors declare no potential conflicts of interest.

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References

1. Shafirstein et al. Br J Cancer. 2018;119(10):1191-9.

> IL123. Invited Lecture
Symposium PDT-6 PDT and oxygen (Theresa Busch)

EFFECTS OF PHOTODYNAMIC THERAPY WITH REDAPORFIN ON TUMOR OXYGENATION AND BLOOD FLOW IN LUNG AND MELANOMA MOUSE MODELS

Authors: Janusz M. Dąbrowski[1*], Malwina Karwicka[2], Barbara Pucelik[1], Martyna Krzykawska-Serda, Michał Gonet[2], Luis G. Arnaut and Martyna Elas[2]
Presenting Author: Janusz M. Dabrowski
1) [1]Faculty of Chemistry and [2]Biophysics Department, Jagiellonian University, Kraków, Poland

Introduction
Redaporfin is a bacteriochlorin-based photosensitizer which is currently in phase II clinical trials. It generates singlet oxygen and hydroxyl radical simultaneously under NIR irradiation.[1-2] Photodynamic therapy strongly affects tissue oxygen levels. Partial oxygen pressure in the PDT-treated tumors changed because of oxygen consumption during PDT, as well as due to fluctuations in oxygen transport after PDT. Similarly, microcirculatory blood flow varies as a result of the disruption of blood vessels due to the treatment.[3]

Methods
LLC and S91/I3 tumors were grown in C57bl or DBA/2 mice respectively. Redaporfin was i.v. administrated and after 15 min (V-PDT) or 3 h (E-PDT) and 72 h (C-PDT) tumors were irradiated with the 750 nm laser. Effects on the vasculature were investigated by: USG with Doppler and PW mode (VEVO 2100), LDPI, EPR oximetry and ELISA.

Results
All PDT protocols examined led to tumor vasculature shut-down and endothelial cells destruction, likely leading to tumor hypoxia as evidenced by VEGF production. Extremely low pO_2 lasting for several days (0-2 mm Hg, i.e. chronic, extreme hypoxia) after vascular-targeted PDT favor long-term tumor responses, in contrast to mild and transient hypoxia, that in tumor-cell targeted PDT lead to strong pO_2 compensatory effects (up to 10-12 mm Hg) and frequent tumor re-growths. V-PDT with redaporfin in the mouse melanoma model provided significant survival advantage, with a cure rate of 44%. Also a significant difference in survival was observed between animals bearing LLC tumors treated with a 3 h DLI protocol (25% of animals without tumor regrowth) and a 15 min DLI protocol (67% cure rate).

Conclusions
Our studies confirm that a key factor for efficient PDT is a complete closure of tumor vessels. In order to achieve this outcome, short DLI protocols should be applied. Application of protocols with longer DLIs usually allow higher PS accumulation in tumor compared to healthy tissue, due to hypoxic conditions in tumors and an unfavorable diffusion barrier assisted by change of the tumor microenvironment, which may lead to tumor reemission and decreased efficacy. Thus, due to tumor hypoxia, PS acting via Type I photochemical mechanisms, which are less independent on O_2 level, should be used in V-PDT.

Acknowledgements
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References

1. Dąbrowski, J. M. *et al Free Radic. Biol. Med.* **52**: 1188-1200; 2012
2. Dąbrowski, J. M. *et al. Coord. Chem. Rev.* **325**, 67-101 (2016).
3. Krzykawska-Serda, M. Dąbrowski J.M. et al. *Free Radic. Biol. Med.* **73**, 239-251 (2014)

> OC045. Oral Communication
Symposium PDT-6 PDT and oxygen (Theresa Busch)

STUDY OF THE RELATIONSHIP BETWEEN RNASET2 AND OXIDATIVE STRESS INDUCED BY PDT

Authors: Enrico Caruso[1], Miryam Chiara Malacarne[1], Stefano Banfi[1], Marzia Bruna Gariboldi[1], Annarosaria de Vito[1], Francesco Acquati[1]
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Introduction

Photodynamic therapy (PDT) is a highly selective and low-invasive therapy for the treatment of solid tumors. The PDT involves the use of a photosensitizing molecule (PS) in association with light of an appropriate wavelength. In the presence of molecular oxygen (O_2), following a series of energy transfers, reactive oxygen species (ROS) are produced, among which there is also the singlet oxygen ($^1O_2$). This species of oxygen has a high level of cytotoxicity and causes most of the cell damage induced by PDT. ROS produced in this way leads to cell death by apoptosis, necrosis or autophagy. In several cell lines, the *RNASET2* gene is correlated to an increase in mortality following the induction of stresses such as lack of amino acids or hypoxia [1]. Thus, this work aims to verify if *RNASET2* could also influence the stress induced by PDT.

Material and Methods

To this purpose, OVCAR-3 cells (deriving from ovarian adenocarcinoma) were used, which differ in the expression of the gene in analysis (expressing the *RNASET2* gene and its silenced counterpart, which is characterized by a reduced expression of the gene under study). In addition to this, the effect of the recombinant *RNASET2* glycoprotein (deriving from *Pichia pastoris*), added to the medium at different concentrations and time, has also been verified. The tests were performed with a PS belonging to the BODIPY family, compounds generally used as fluorescent dyes [2] that can be modified with the introduction of iodine atoms thus becoming an alternative class of PSs [3], which has recently found application in photodynamic therapy [4]. To gain some insights into the mechanism of PS-induced phototoxicity, induction of apoptotic, autophagic and necrotic cell death, and generation of reactive oxygen species (ROS) were evaluated in cancer cells following exposure to the PSs and irradiation. The effect of the PSs on the migratory activity of the cells was also assessed.

Results and Discussion

The results obtained confirm that the *RNASET2* gene leads to an increase in cellular mortality under the stress induced by PDT; however this result was not replicable following the addition of the recombinant glycoprotein in the cell culture.

Conflict of interest

The authors declare that they have no conflict of interest.

Reference

[1] M. Lualdi, E. Pedrini, K. Rea, L. Monti, D. Scaldaferri, M. Gariboldi, A. Camporeale, P. Ghia, E. Monti, A. Tomassetti, F. Acquati, Roberto Taramelli Oncotarget 6 (10) (2015) 7851-7865
[2] Y. Nakamura, H. Makino, D. Citterio, K.J. Suzuki, J. Am. Chem. Soc. 130 (2008) 1550–1551.
[3] T. Yogo, Y. Urano, Y. Ishitsuka, F. Maniwa, T. Nagano, J. Am. Chem. Soc. 127 (2005) 12162
[4] E. Caruso, M. Gariboldi, A. Sangion, P. Gramatica, S. Banfi J. Photochem Photobiol B: Biol. 167 (2017) 269-281

> P080. Poster

Symposium PDT-6 PDT and oxygen (Theresa Busch)

PHOTODYNAMIC ACTIVITY OF NEW PHOTOSENSITIZERS OBTAINED FROM 5,10,15,20-TETRAPENTAFLUOROPHENYLPORPHYRIN

Authors: Enrico Caruso[1], Stefano Banfi[1], Miryam Chiara Malacarne[1]
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Introduction

Photodynamic therapy (PDT) is an alternative or adjuvant treatment to classical cancer therapies which achieve the goal to kill cancer cells by using non-toxic drugs or dyes (photosensitizers) that are pharmacologically active only after exposure to light in the presence of oxygen [1]. The four mainly used molecular structures of photosensitizers belongs to the class of porphyrin, chlorins, phthalocyanines, and porphycenes derivatives [2].

Material and Methods

The commercially available tetrapentafluorophenylporphyrin has been used as parent compounds for the synthesis of six new tetraarylporphyrins. These new porphyrins were isolated as pure compounds after column chromatography purification, following nucleophilic substitution of the *para*-position fluorine by means of oxygen and sulphur anion, providing either tri- or tetra-substituted derivatives. Of these new porphyrins, were first determined the photobleaching stability and the octanol/water repartition values (LogP), and then were studied as photosensitizers (PSs) against HCT116 cancer cell line irradiating with a blue LED device.

Results and Discussion

The intrinsic toxicity of all these compounds was negligible whereas the photodynamic efficacy was found related to the hydrophilicity of the tethered moiety as the hydroxy substituted compound was found to be the more efficient compared with the methoxy substituted derivatives. On the contrary, the PSs lacking of any polar groups were found poorly efficient.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Robertson CA, Evans DH, Abrahamse H (2009) Photodynamic therapy (PDT): a short review on cellular mechanisms and cancer research applications for PDT. J Photochem Photobiol B 96:1-8
[2] Robertson CA, Stockert JC, Canete M, Juarranz A (2007) Porphycenes: facts and prospects in photodynamic therapy of cancer. Curr Med Chem 14:997-1026

> P081. Poster

Symposium PDT-6 PDT and oxygen (Theresa Busch)

EVALUATION OF HYPERICIN BASED ION PAIR ON PHOTODYNAMIC ACTIVITY IN VMM 39 MELANOMA CELL LINE

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Presenting Author: Gislaine Patricia de Andrade
1) Federal University of São Paulo 2) Federal University of ABC

Introduction

Skin cancer is one of the most common types of cancers in the world population and one of the most frequent in Brazil, due to the great solar incidence in the country. Melanoma is the most lethal type of skin cancer and has increased incidence over the past few decades. Some therapies have being used in the treatment of melanoma, such as drugs targeting the BRAF protein and inhibitory drugs in MEK, but they are liable to cause severe side effects to the patients. Photodynamic Therapy (PDT) comprises administering a photosensitizer, which, after accumulation in the affected tissue, is exposed to light of specific wavelength and generates reactive oxygen species (ROS), which are capable of triggering cell death by specific reactions. PDT can be used in the treatment of cancer and has shown fewer side effects when compared to other antineoplastic therapies. Hypericin is currently considered as a third generation photosensitizer, due to its chemical characteristics, the fact that it changes from its monomeric state to its aggregate state when in biological medium, thus reducing its photodynamic effects. In this case, molecular alterations to decrease the state of aggregation are necessary for this compound to be applicable in medical therapy.

Objective

The present study aims to evaluate the photodynamic effects of hypericin and hypericin ion pair (HYP-glu) on melanoma VMM 39 cell line.

Methods

Among the strategies which will be used are evaluation of the photodynamic effect on the viability and clonogenicity of melanoma tumor cells. Possible alterations of the extracellular matrix will be analyzed by expression of heparanase and proteoglycans.

Results

Preliminary results showed the effectiveness of the photodynamic activity was higher for the hyp-Glu, when compared to the hypericin, in human breast cancer. The molecular modifications in the hypericin macrocycle caused changes in the interaction between photosensitizers and the cells. In order to highlight the potential use of PDT as an alternative treatment for melanoma as well as elucidate important molecular mechanisms and extracellular matrix changes in carcinogenesis, comparative studies will be carried out between breast cancer and melanoma.

> P082. Poster

Symposium PDT-6 PDT and oxygen (Theresa Busch)

PHOTOINDUCED CANCER CELLS OXIDATION STRESS GENERATING BY NOVEL BISCYANINE DYE

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Among a wide variety of cyanine dyes (CD) biscarbocyanine dyes occupy a special place (BCD). Here we report a synthesis and properties of the BCD series with different substituents (n= 1, 2 ; R1, R2 = CH_3, $(CH_2)_5CH_3$, $(CH_2)_4SO_3$, CH_2COOMe, $(CH_2)_{10}COOH$, $(CH_2)_{10}COOEt$, $(CH_2)_4P(O)(OEt)_2$, $(CH_2)_4SO_3$). The interaction between two coupled chromophore systems leads to the splitting of the singlet state energy level and the appearance of the absorption band in the near infra-red region of the spectrum. Comparing to single-chromophore dyes, BCD has a higher yield of intersystem crossing. BCD demonstrate the ability to accumulate in cancer cells, its fluorescence in the NIR range effectively passes through the tissues and allows imaging.

Spectral and photochemical properties in aqueous and organic media were studied for the synthesized dyes. Due to the triplet state formation under IR irradiation compound BCDC was chosen. Radical anion BCDC was formed as a result of the electron phototransfer reaction from the donor, N,N-dimethylaniline, to the dye's triplet state. Fluorescence of the dye in cells was registered. Photoexcitation of BCDC in cancer MCF7 cells leads to superoxide radical anion generation. The formation mechanism of superoxide anion includes phototransfer of electron from intracellular protein structures to the BCDC triplet state, which leads to oxidative stress in cells. The formation of BCDC complexes with albumin increases the quantum yield of dye fluorescence, and its mechanism was determined by spectral-kinetic methods. The possible interactions between the BCD molecule and HSA (PDB: 4L9Q) were analyzed by means of semirigid molecular docking. Synthesized BCDC can be considered as a promising compound for theranostics.

Acknowledgements

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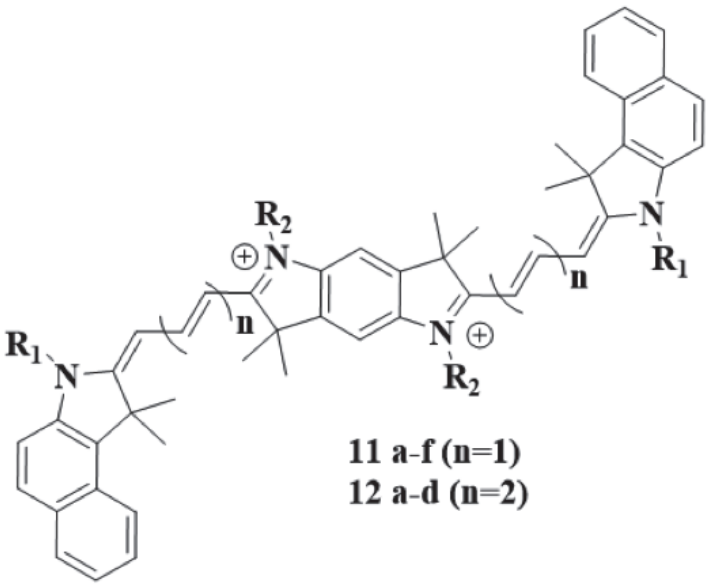

> P083. Poster

Symposium PDT-6 PDT and oxygen (Theresa Busch)

HUMAN LEUKEMIA CELLS PHOTOINACTIVATION EMPLOYING AN ANTHRAQUINONE FROM ARGENTINIAN FLORA

Authors: María Laura Mugas[1,2], Gustavo Calvo[1], Juliana Marioni[2], Pablo Vallecorsa[1], Mariela Céspedes[1], Daniel Sáenz[1], Gabriela di Venosa[1], Susana Núñez Montoya[3], Adriana Casas[1]
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Parietin (PTN), an anthraquinone (AQ) found in some vegetal species even lichens, has been shown to be a good photosensitizer with promising applications in bacterial photoinactivation[1]. The aim of this work was to evaluate the *in vitro* activity of PTN as photosensitizer on K562 human leukemic cells; in order to estimate its potential use in Photodynamic Cancer Therapy (PDT).

PTN (1,8-dihydroxy-3-methoxy-6-methylanthraquinone) was isolated from the lichen *Teoloschistes nodulifer* (Nyl.) Hillman (Teloschistaceae) and it was purified by recrystallization from the acetone extract, and its purity was determined by HPLC.

Employing human leukemic K562 cells, we determined: a) PTN maximum non-cytotoxic concentration (MNCC on darkness conditions)[2]; b) incorporation time (1 h-24 h); c) incorporation mechanism (passive or active transport); d) LD_{50}: light dose inducing 50% of cell death after PDT treatment (MNCC of PTN, irradiation time ≤ 30 min) and e) cell cycle analysis after PDT in order to estimate the cell death mechanism. The results of experiments a) to d) were obtained by means of cellular viability measure, by employing the MTT colorimetric assay[3], and experiment e) by flow cytometry analysis, using propidium iodide staining. K562 cells were used at semi confluency, PTN was prepared in RPMI medium with DMSO ≤ 1% and the irradiation doses were adjusted employing different times of exposition to a light system, which consisted in 2 blue compact fluorescent lamps (Sica, 15 W).

PTN (purity of 91.2 ± 0.2%) presented a MNCC of 30 µM on K562 cells. Since little difference was observed between 1 h and 24 h incorporation, the optimal incubation time of PTN was set as 1 h. Passive transport seems to be the main mechanism involved in PTN entry to the cells, since not significant differences were observed between incorporation at 4 and 37°C. After illumination of K562 cells exposed to PTN, the LD_{50} was 1,39 J/cm^2 (5 min), and cell cycle analysis suggested that apoptosis was involved in PTN-PDT treatment (55 %). Therefore, this natural AQ produced photo-destruction of leukemic cells, at non- cytotoxic concentrations employing visible light.

The results of this work confirm the potential use of parietin in PDT, supporting the recommendations of the World Health Organization to revalue phytomedicine and consider the healing properties of the country\'s flora. Currently, we are carrying out studies of PTN-PDT on cell lines of solid tumors, as well as in non-tumor cells.

References

1- Comini L., Morán Vieyra F., Mignone R., et al., Photochem. Photobiol. Sci., 16, 201, 2017.
2- Liu A., Shu S., Qin H., Lee S., Wang Y., Du G., Planta Med., 75, 137, 2009.
3- Denizot F, Lang R., J. Immunol. Methods., 89, 271, 1986.

> P084. Poster

Symposium PDT-6 PDT and oxygen (Theresa Busch)

STROMA-RICH CO-CULTURE MULTICELLULAR TUMOR SPHEROIDS AS A RELEVANT IN VITRO MODEL HEAD AND NECK SQUAMOUS CANCER FOR SCREENING OF PHOTOACTIVE DRUGS

Authors: Ilya Yakavets[1,2,3], Samuel Jenard[2,4], Aurelie Francois[2], Yulia Maklygina[1,2,5], Victor Loshenov[5,6], Henri-Pierre Lassalle[1,2], Gilles Dolivet[1,2], Lina Bezdetnaya[1,2]
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Introduction

Physiologically relevant *in vitro* cellular models are required to make more reliable the preclinical studies to test drug efficacy. These models should recapitulate the morphology, microenvironment, cell-cell and cell-stroma interactions existing in solid tumors. Stroma components as fibroblasts and macrophages are essential components of head and neck cancer microenvironment. The present study was aiming at developing a 3D co-culture model of head & neck squamous cell carcinoma cells and stromal fibroblasts cells to better mimic *in vivo* tumor microenvironment.

Results and discussions

We constructed 3D multicellular spheroids consisting of FaDu pharynx squamous cell carcinoma tumor cells and MeWo cancer-associated fibroblasts (CAF). The developed spheroids were optimized, characterized by fluorescence microscopy and immunohistochemical analysis of spheroid cryo-section and appeared to reproduce sufficiently a stroma-rich head and neck carcinoma tumors. and neck carcinoma tumors and could significantly help in anticancer drug screening. The generated co-culture FaDu/MeWo spheroids were applied for studies of penetration, diffusion and antitumor efficacy of photoactive drugs used in the photodiagnosis and photodynamic therapy.

Conclusions

We successfully constructed 3D co-culture multicellular tumor spheroids consisted of tumor cells and cancer-associated fibroblasts mimicking *in vivo* microenvironment. The data obtained confirm the interest of co-cultured hetero-spheroids for the screening of photoactive drugs.

Acknowledgments

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> P085. Poster

Symposium PDT-6 PDT and oxygen (Theresa Busch)

IN VITRO EVALUATION OF PHOTODYNAMIC ACTIVITY OF THE NOVEL HYDROPHILIC AND AMPHIPHILIC ANIONIC (AZA)PHTHALOCYANINE DERIVATIVES FOR TREATMENT OF TUMOROUS DISEASES

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Phthalocyanines (and their nitrogen analogues – azaphthalocyanines) proved to be a very promising second generation photosensitizers (PS) for application in cancer therapy receiving increased attention in the last few years. These are synthetic macrocyclic dyes with optimum photophysical properties, characterized mainly by high quantum yield of $^1O_2$, strong absorption at longer wavelengths and very low inherent toxicity. The anionic water-soluble zinc(II) phthalocyanines with sulfonyl or carboxyl substituents were synthesized. The aim of this work was to evaluate the photodynamic activity of those newly synthesized hydrophilic and amphiphilic anionic PSs from the group of (aza) phthalocyanines at the cellular level under *in vitro* conditions and based on the results to assess their cytotoxic effect.

Cytotoxicity experiments were performed mainly on human cervix carcinoma cell line HeLa using neutral red uptake assay on 96-well plates. The toxicity experiments were performed after irradiation as well as in the absence of activating light. Uptake profiles of PSs to the cells were determined by measuring fluorescence in the cell lysate. Furthermore, localization of the compounds within the cell (cell membrane, mitochondria, lysosomes, nucleus, etc.) and detection of morphological changes after PS activation at the level of whole cells and subcellular structures were studied by using a fluorescence and confocal laser scanning microscopy.

The results of individual experiments on HeLa cells have shown high photodynamic activity after irradiation and very low inherent toxicity of all studied compounds. The most suitable properties were achieved with compounds HK22Zn (EC_{50} = 5.4 μM, TC_{50} > 1000 μM) and P44Zn (EC_{50} = 0.33 μM, TC_{50} > 1000 μM) in the cell culture medium from the hydrophilic and amphiphilic group, respectively. Photodynamic activities of studied PSs were strongly influenced by the presence of serum in the cell culture medium lowering the activity approx. ten and hundred times for HK22Zn and P44Zn respectively. Uptake of the PSs into the cancer cells was rapid in the first two hours than reaching stead-state. Tested compounds were found to localize intracellularly, mainly within the lysosomes thus suggesting an endocytic mechanism of cellular uptake. Amphiphilic compounds were also found in the cell membrane. For all examined compounds, photodynamic effect of PS resulted in significant morphological changes indicating ongoing cell death.

In summary, we have prepared and *in vitro* investigated novel anionic phthalocyanines. Based on obtained results, studied compounds proved to be interesting PSs for the photodynamic therapy of tumorous diseases. Selected compounds will be included in subsequent studies on 3D spheroid cultures as well as in the *in vivo* evaluation of their photodynamic efficiency on mouse tumour model.

The work was supported by Grant Agency of Charles University No. 1620219, Czech Science Foundation 1914758Y and SVV 260 416.

> P086. Poster

Symposium PDT-6 PDT and oxygen (Theresa Busch)

PHOTOCHEMICAL AND PHOTOPHYSICAL STUDIES OF BIOSUPRAMOLECULAR COMPLEX OF HUMAN ALBUMIN SERUM, CUCURBITURILS AND TOLUIDINE BLUE DERIVATE

Authors: Nory Mariño-Ocampo[1], Denis Fuentealba[1]
Presenting Author: Nory Mariño-Ocampo
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Photodynamic therapy (PDT) is an emerging therapeutic treatment for cancer. PDT, which is a minimally invasive process, requires the use of a molecule called photosensitizer (PS), which contains a natural or synthetic chromophore that absorbs light of a specific wavelength. This PS is incorporated into the tumoral tissue and is then excited by a source of light to generate reactive oxygen species (ROS) that are aimed to destroy tumoral cells.1 One of the most important challenges of this therapy is the development of new PS, that present better selectivity, and for that reason our research is focused on the use of biosupramolecular complexes as drug delivery systems.

The advantages of using proteins as carriers of different molecules are well reported in the literature.A useful protein to develop PS carriers is human serum albumin (HSA).2 HSA has been shown to enhance the selectivity of different cancer drugs and different PS.3,4 On the other hand, cucurbiturils (CB[n]s) are a family of macrocyclic compounds formed by units of glycoluril of different sizes (5-8,10), joined by methylene bridges cyclically, forming hydrophobic cavities. Several complexes between PS and CB[n]s have been reported.5 It has been shown that complexation of PS with CB[n]s is a good option to control the photochemical and photophysical properties of the molecules and their interactions with biomolecules.6

In the present work, we propose to develop biosupramolecular complexes with a toluidine blue (TBO+) derivative that is covalently conjugated with HSA and has good binding affinity towards CB[7]. Specifically, we will present results on the photophysical properties of the complexes such as absorption and emission spectra, time-resolved fluorescence, photostability and singlet oxygen generation.

References

(1) Chen, Y.; Liu, J.; Song, M.; Jiang, L.; Liu, L.; Liu, Y.; Fu, G.; Xue, J.; Liu, J.; Huang, M.; et al. Insights into the Binding Mechanism of BODIPY-Based Photosensitizers to Human Serum Albumin: A Combined Experimental and Computational Study. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2018**,
(2) Absar, S.; Nahar, K.; Choi, S.; Ahsan, F.; Yang, V. C.; Kwon, Y. M. Serum Albumin–Protamine Conjugate for Biocompatible Platform for Targeted Delivery of Therapeutic Macromolecules. *J. Biomed. Mater. Res. Part A* **2014**,
(3) Kratz, F.; Elsadek, B. Clinical Impact of Serum Proteins on Drug Delivery. *J. Control. Release* **2012**, *161* (2), 429–445.
(4) Oluwole, D. O.; Prinsloo, E.; Nyokong, T. Photophysical Behavior and Photodynamic Therapy Activity of Conjugates of Zinc Monocarboxyphenoxy Phthalocyanine with Human Serum Albumin and Chitosan. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2017**, *173*, 292–300.
(5) Robinson-Duggon, J.; Pérez-Mora, F.; Dibona-Villanueva, L.; Fuentealba, D. Potential Applications of Cucurbit[n]Urils Inclusion Complexes in Photodynamic Therapy. *Isr. J. Chem.* **2017**, *58* (3–4), 199–214. https://doi.org/10.1002/ijch.201700093.
(6) Assaf, K. I.; Nau, W. M. Cucurbiturils: From Synthesis to High-Affinity Binding and Catalysis. *Chem. Soc. Rev.* **2015**, *44* (2), 394–418.

> IL124. Invited Lecture

Symposium PDT-7 Immunology in PDT (Mladen Korbelik)

RAPID TUMOR ABLATION THERAPIES INVOLVE COMMON STRESS RESPONSE SIGNALING NETWORKS AND PROMOTION OF IMMUNE RESPONSE AGAINST TREATED CANCERS

Authors: Mladen Korbelik[BC Ca]
Presenting Author: Mladen Korbelik
1) BC Cancer, Vancouver BC, Canada

Oxidative stress inflicted in targeted cancer cells by photodynamic therapy (PDT) and corresponding thermal stress from treatment with photothermal therapy (PTT), cryoablation therapy (CAT) or other thermal-based tumor ablation modalities trigger common threat of proteostasis impairment. This evokes in cells treated by all these therapies evolutionary well preserved canonic protection mechanism operated by stress signaling networks. For instance, PDT response includes heat shock signaling, heme regulator inhibitor kinase-mediated integrated stress response signaling, antioxidant response signaling, and p53-mediated stress signaling. The unfolded protein response signaling pathways as well as ER-associated protein degradation (ERAD) pathway were found to be engaged following PDT, PTT and CAT treatments. Another shared event with rapid tumor ablation therapies is the induction of immunogenic cell death (ICD) orchestrated by a massive liberation of highly immunogenic tumor antigens with coordinate extensive vigorous immunostimulatory signaling. Such occurrence enables these therapies to generate a potent immune response against treated cancers. To translate this elicited development into a robust and long-lasting antitumor efficacy in clinical setting it is often critical to enlist into the combination an optimal immunoadjuvant. Featured will be one promising candidate for such agent, N-dihydrogalactochitosan (GC).

> IL125. Invited Lecture
Symposium PDT-7 Immunology in PDT (Mladen Korbelik)

PHOTODYNAMIC THERAPY IN THE ERA OF CANCER IMMUNOTHERAPY

Authors: Jakub Golab[1]
Presenting Author: Jakub Golab
1) Department of Immunology, Medical University of Warsaw, Poland

A major challenge in oncology is to develop effective therapeutic strategies to target advanced cancer. Recent therapeutic options for cancer patients have significantly broadened by approvals of various types of immunotherapies. The possibility to stimulate the immune response against cancer has raised enormous hopes among all stakeholders. Initial results from clinical studies reported spectacular antitumor efficacy. However, meta-analyses from increasing number of larger scale trials have slightly tempered this enthusiasm and revealed that still a vast majority of patients do not benefit from cancer immunotherapy to a desired extent. This prompts the scientific community to further develop immunotherapeutic approaches and to look for combination treatments that might be safe and more effective.

Photodynamic therapy (PDT) is a clinically approved therapeutic procedure used for the management of various types of solid tumors and nonmalignant diseases. The principle of PDT in cancer treatment is based on local or systemic administration of photosensitizing chemical compound (photosensitizer) followed by light illumination of the tumor and surrounding normal tissue. Light-triggered photoactivation of PS initiates a physico-chemical process leading to generation of singlet oxygen and secondary reactive oxygen species that cause microdamage to subcellular biomolecules. Since the pioneering works of Korbelik et al. that revealed the importance of innate and adaptive immunity in the therapeutic effects of PDT, an increasing number of studies report various types of immunotherapeutic approaches used to potentiate antitumor effects of this treatment. Coincidentally, therapeutic effects of the combined PDT+immunotherapy approaches are mitigated by adaptive processes associated with tumor development or PDT itself. Thus, based on accumulating data as well as our preliminary results the molecular mechanism involved in the development of these adaptive processes to improve immunotherapeutic outcomes of photodynamic therapy will be discussed.

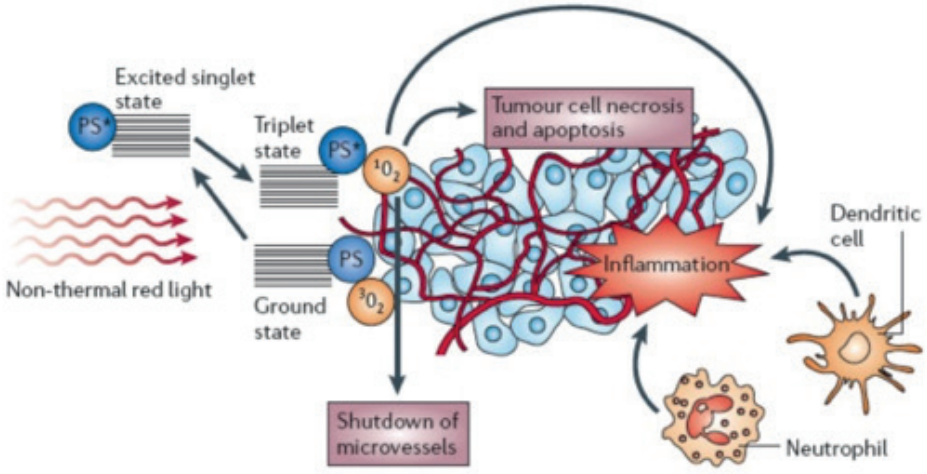
> IL126. Invited Lecture

Symposium PDT-7 Immunology in PDT (Mladen Korbelik)

STRATEGIES TO POTENTIATE IMMUNE RESPONSE AFTER PHOTODYNAMIC THERAPY

Authors: Michael Hamblin[1949]
Presenting Author: Michael R Hamblin
1) Massachusetts General Hospital

It has been known for decades that in some cases (but not all) photodynamic therapy (PDT) can induce a potent systemic immune response against cancer that may allow distant metastases to be destroyed by a local treatment. This has been shown both in many small laboratory animal models of cancer and also in a limited number of clinical scenarios. However the determinants of this phenomenon remain little understood. We have obtained evidence that a very important factor is whether or not the tumor expresses antigens that can be broadly described as “tumor rejection antigens”. These antigens can be presented to naïve T-cells by dendritic cells (DC) allowing the formation of antigen-specific cytotoxic T-cells that can track down and destroy distant tumors. There are several strategies that may be combined with PDT to increase the likelihood and the strength of this response. One of the most investigated approaches is to use various ligands that can bind to and stimulate DC and other antigen-presenting cells. These ligands can include such agents as toll-like receptor (TLR) agonists including CpG oligonucleotides that activates TLR9 and C-type lectin agonists such as glycated chitosan. Another powerful strategy is the use of agents which deplete regulatory T-cells (CD4+CD25+foxp3+) that are responsible for suppressing the both priming phase and also the effector phase of the anti-tumor immune response. These agents may include the clinically applicable drug low-dose cyclophosphamide.



> IL127. Invited Lecture
Symposium PDT-7 Immunology in PDT (Mladen Korbelik)

MODELING SURGERY-INDUCED INFLAMMATION AS AN EFFECTOR OF ADJUVANT THERAPY

Authors: Theresa Busch[1]
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Introduction
Photodynamic therapy (PDT) offers a means to eradicate residual disease after surgical debulking. PDT is being studied in treatment of malignant pleural mesothelioma (MPM) as intraoperative delivery after macroscopic complete resection. In this context, PDT is delivered in the peri-operative environment, which is expected to be highly inflamed and thus may result in associated effects on adjuvant therapy.

Methods
In a clinical trial of intraoperative PDT for MPM, serum levels of cytokines were measured at multiple timepoints in relation to the start of surgery. Similarly, cytokine levels were measured in murine mesothelioma tumors exposed to either tumor resection or surgical insult ("tumor incision" model[1]) (Fig. 1). Using the tumor incision (TI) model, we determined the effects of a surgical insult on tumor properties during PDT and the antitumor immunity that it generated.

Results
In both clinical studies and murine models, surgery was associated with increases in inflammatory cytokines such as IL-6 over the hours after first incision. Irrespective of the presence of surgery-induced inflammation, the benefit of combining surgery and PDT was clearly identifiable in murine models. The addition of PDT to surgical debulking provided for a complete response that was not achievable by either modality alone. Nevertheless, potential negative effects of inflammation on PDT outcome could be identified. These were not a function of tumor photosensitizer uptake, oxygenation, or light propagation. However, aspects of PDT-initiated immunity were altered in the TI model.

Conclusions
The addition of PDT to surgical debulking provides for better long-term outcome in preclinical models of mesothelioma. Surgery-induced inflammation can be introduced in murine models over a timeframe similar to that found in clinical application, and not surprisingly, the insult of surgery can attenuate response to adjuvant therapy. Given the overall benefit of combining surgery with adjuvant therapies such as PDT, it is valuable to discern the mechanisms of this interaction to identify points of intervention.

References

[1] Davis RW 4th, et al. *A preclinical model to investigate the role of surgically-induced inflammation in tumor responses to intraoperative photodynamic therapy*. Lasers Surg Med. 2018 Jul;50(5):440-450. doi: 10.1002/lsm.22934.

> IL128. Invited Lecture
Symposium PDT-7 Immunology in PDT (Mladen Korbelik)

T CELL-BASED IMMUNO-PHOTODYNAMIC THERAPY OF CANCER

Authors: Ferry Ossendorp[LUMC]
Presenting Author: Ferry Ossendorp
1) Leiden University Medical Center

We studied local and systemic immune effects of Photodynamic therapy (PDT) of established tumors. In four independent aggressive mouse tumor models, we combined PDT with two types of T cell-based immunotherapy: specific immunotherapy by vaccination with synthetic peptides containing T cell epitopes from known tumor antigens and non-specific therapy checkpoint blocking antibodies. We show that these immunotherapies can be efficiently combined with PDT to eradicate established tumors, based on strong local tumor ablation and the induction of a robust systemic immune response. Combination treatment of PDT with therapeutic SLP vaccination cured one third of mice. Importantly, all cured mice were fully protected against subsequent tumor rechallenge, and combination treatment of primary tumors led to eradication of distant secondary tumors, indicating the induction of a systemic antitumor immune response. Combination therapy of PDT and CTLA-4 blocking antibodies significantly improved therapeutic efficacy and survival of double-tumor-bearing mice. These results show that local tumor ablation by PDT induces CD8 T cell responses crucial for systemic tumor eradication, which can be further enhanced by combination with immune checkpoint blockade. This last strategy could be a novel therapeutic intervention for advanced cancer without previous knowledge of tumor-specific antigens. Our results suggest that combination of active immunotherapy with tumor ablation by PDT is a clinically feasible approach.

References

Kleinovink JW, van Driel PB, Snoeks TJ, Prokopi N, Fransen MF, Cruz LJ, et al. Combination of Photodynamic Therapy and Specific Immunotherapy Efficiently Eradicates Established Tumors. 2016 Clin Cancer Res 22:1459-68.

Kleinovink JW, Fransen MF, Löwik CW, Ossendorp F. Photodynamic-Immune Checkpoint Therapy Eradicates Local and Distant Tumors by CD8(+) T Cells. 2017 Cancer Immunol Res. 5:832-838.

> OC046. Oral Communication
Symposium PDT-7 Immunology in PDT (Mladen Korbelik)

PDT-INDUCED PROSTAGLANDINE2 PLAYS AN UNEXPECTED BENEFICIAL ROLE IN THE GENERATION OF ANTI-TUMOR IMMUNITY

Authors: Riddhi Falk-Mahapatra[1], Sandra Gollnick[1]
Presenting Author: Riddhi Falk-Mahapatra
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Introduction
Blockade of Prostaglandin E2 (PGE2) reduces chronic inflammation associated with cancer and can improve the efficacy of cancer immunotherapy. However, the enhancement of anti-tumor immunity by Photodynamic Therapy (PDT) is dependent upon acute inflammation, which is characterized by neutrophil infiltration to tumor-draining lymph nodes (TDLNs). PGE2 is also a mediator of acute inflammation; however, its role in the enhancement of anti-tumor immunity by PDT is uncertain. We hypothesized that acute expression of PGE2 plays a beneficial role in enhancing anti-tumor immunity by PDT.

Methods
Our studies employed murine models of colon carcinoma and head and neck cancer. PGE2 kinetics in tumor and TDLNs were assayed at 0, 4 and 24 hours after PDT by ELISA. To determine the role of acute expression of PGE2 in the regulation of PDT-induced acute inflammation, enhancement of anti-tumor immunity, and overall PDT efficacy, we blocked the PGE2 synthesis pathway. This was achieved by blocking COX2, the key enzyme in PGE2 synthesis pathway, by a single administration of its inhibitor NS398 immediately prior to PDT.

Results
Our studies confirm acute expression of PGE2 after PDT in line with PDT-induced acute inflammation. Administration of NS398 eliminated the treatment-induced PGE2 surge and resulted in significantly faster tumor regrowth to endpoint. These mice had reduced expression of pro-inflammatory cytokines in TDLNs and infiltration of neutrophils to TDLNs following PDT. We also observed reduced accumulation of activated dendritic cells in the TDLNs in response to PDT along with reduced numbers of activated CD8 T cells.

Conclusion
Taken together, our results indicate that PDT generates a PGE2 surge that regulates treatment-induced acute inflammation. This PGE2 surge is beneficial for PDT-enhanced anti-tumor immunity and overall PDT efficacy. Current standard treatment in clinical PDT involves NSAID administration both pre and post PDT. Our preclinical findings suggest the acute burst of PGE2 immediately after PDT is beneficial for the induction of anti-tumor immune response, which has significant clinical implications.

> OC047. Oral Communication

Symposium PDT-7 Immunology in PDT (Mladen Korbelik)

EXPLORING THE POTENTIAL FOR IMMUNE MODULATION TRIGGERED BY NANOBODY-TARGETED PHOTODYNAMIC THERAPY

Authors: Irati Beltrán Hernández[1], Yingxin Yu[2], Tommaso del Buono D'Ondes[1], Alessia di Maggio[2], Sabrina Oliveira[1,2]
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Introduction

Nanobody-targeted photodynamic therapy (NB-PDT) has been developed as a potent and more selective approach for cancer therapy, compared to conventional PDT [1]. It has been shown that conventional PDT is able to induce immunogenic cell death, characterized by the exposure/release of damage associated molecular patterns (DAMPs) from dying tumor cells, and leading to anti-tumor immune responses [2]. In this study, we aim at understanding the possible immune modulation triggered by NB-PDT.

Methods

The photosensitizer IRDye700DX was conjugated to the EGFR-targeted NB 7D12 and used to perform NB-PDT on EGFR-overexpressing A431 tumor cells. After 30 min incubation with the NB-PS conjugates, A431 cells were exposed to a light dose of 10 J/cm^2. Intracellular localization of DAMP HSP70 and HMGB1 were assessed on treated cells by immunofluorescence. HSP70, ATP and inflammatory cytokines (i.e. IL-1β, IL-6 and IL-8) were quantified in the supernatants from treated tumor cells. Furthermore, human monocyte derived dendritic cells (moDC) were generated and co-incubated with treated tumor supernatants for 24 hrs. DC maturation marker CD86 and MHCII were then analyzed by flow cytometry.

Results

The cytoplasmic DAMP HSP70 was detected on the cell membrane after mild NB-PDT (1 nM conjugate), while it was detected in the supernatant after highly cytotoxic NB-PDT (25 nM conjugate). The nuclear DAMP HMGB1 was found in the cell cytoplasm under both NB-PDT conditions. Furthermore, cells treated with highly cytotoxic NB-PDT showed an increased release of ATP and pro-inflammatory cytokines IL-1β and IL-6, whereas pro-tumoral IL-8 release was decreased. Lastly, supernatants collected from tumor cells treated with highly cytotoxic NB-PDT were able to induce the phenotypic maturation of human moDC, as indicated by the upregulation of CD86 and MHC II on the cell surface.

Conclusions

Altogether, these results point to the immune-modulation by NB-PDT, which can be exploited to increase NB-PDT efficacy even further.

Acknowledgements

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Conflicts of interest

The authors report no conflict of interest.

References

1. van Driel, P., et al., *EGFR targeted nanobody-photosensitizer conjugates for photodynamic therapy in a pre-clinical model of head and neck cancer.* J Control Release, 2016. **229**: p. 93-105.
2. Mroz, P., et al., *Stimulation of anti-tumor immunity by photodynamic therapy.* Expert Review of Clinical Immunology, 2011. **7**(1): p. 75-91.

> P087. Poster

Symposium PDT-7 Immunology in PDT (Mladen Korbelik)

CHANGES OF CELLULAR MORPHOLOGY IN RESPONSE TO PHOTODYNAMIC TREATMENT IN VITRO DIFFER ESSENTIALLY FOR DIFFERENT TUMOR LOCALIZATIONS AND INDIVIDUAL PATIENTS

Authors: Irina Semenova[1], Andrey Belashov[1], Anna Zhikhoreva[1], Natalia Avdonkina[2], Irina Baldueva[2], Anna Danilova[2], Mark Gelfond[2], Tatiana Nekhaeva[2], Oleg Vasyutinskii[1]
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We have recently demonstrated high potentials of digital holographic microscopy (DHM) and tomography (DHT) in studies of cellular response to photodynamic and chemotherapeutic treatment [1-3]. DHM/T provide quantitative information on cellular morphology and optical characteristics, they are label-free and allow for monitoring morphological changes in cells in dynamics. In this communication we present an analysis of morphological changes in melanoma (Mel), soft tissue sarcoma (STS) and renal cell carcinoma (RCC) cell cultures, induced by photodynamic treatment in vitro. Tumor samples were obtained from individual patients during surgery. Cell lines were established after mechanical desegregation and at least 10 passages in culture. For each tumor type experiments were performed on cell lines obtained from 3 patients. Cell samples were incubated in the solution of Radachlorin photosensitizer in culture medium and then irradiated by a semiconductor laser for the induction of intracellular response. After irradiation cell cultures were monitored in the holographic microscope or tomographic microscope during 1.5 hours, measurements were taken every five minutes. Changes in cellular morphology and optical characteristics, including cell volume, membrane area, projected area, dry mass, intracellular distributions of refractive index, were monitored and analyzed. The analysis performed allowed for distinguishing between cell death pathways and dynamics at different irradiation doses. It was shown that cells from different solid tumors demonstrate essentially different response to photodynamic treatment at the same irradiation doses. In particular, Mel cells have shown much higher resistance to photodynamic treatment as compared to RCC and STS cells. Moreover cells of the same tumor localization but taken from different patients also demonstrated substantially different response to treatment at the same doses. The results obtained and the developed methodology can give additional information for analysis of response of patients to PDT and for optimization of PDT protocols for individual patients.

References

[1] Belashov, A.V., et al. “Digital holographic microscopy in label-free analysis of cultured cells’ response to photodynamic treatment.” Optics Letters, 41, 21, 5035-5038, (2016).
[2] Semenova, I.V. et al., Necrosis and apoptosis pathways of cell death at photodynamic treatment in vitro as revealed by digital holographic microscopy,” Proc. SPIE, 10497, 104970D, (2018).
[3] A.A. Zhikhoreva, et al., Morphological changes in the ovarian carcinoma cells of Wistar rats induced by chemotherapy with cisplatin and dioxadet. Biomedical Optics Express, 9, 11, 5817-5827, (2018).

> 129. Invited Lecture
Symposium PDT-8 Excitations in PDT (Celine Frochot)

RADIOLUMINESCENT NANOMATERIALS: TOWARDS DEEP TISSUE PDT ACTIVATION DURING RADIATION THERAPY

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Photodynamic therapy (PDT) has seen long standing interest as a therapy for resistant cancers, but the main Achilles' heel for its successful clinical exploitation is the use of poorly penetrating visible light. Indeed, PDT is intrinsically restricted by the low penetration depth of light in tissue and is therefore mostly used to treat superficial or optical-fiber accessible lesions. An elegant non-invasive approach to overcome this limitation is to conjugate the photosensitizers to radioluminescent nanomaterials, also called nanoscintillators, and to activate these with radiation therapy. Upon X-ray irradiation, nanoscintillators are "switched on" and emit light that can subsequently excite the photosensitizers and induce PDT. As X-rays penetrate deeply in tissues, radioluminescence could activate PDT non-invasively at depth during radiation therapy and without being restricted by large tumor volumes and optical shielding by blood vessels. The heavy-element nanoscintillators can additionally induce a so-called radiation dose-enhancement effect due to Auger cascades and photoelectrons emitted by the nanoparticles upon X-rays irradiation.

Although relatively new, this approach quickly gained interest within the last decade. In this presentation, we will first draw an overview of the X-PDT state of the art. We will also discuss the potential therapeutic contributions that can enhance the overall radiation therapy efficacy and the choice of radiation sources that can be employed.

Acknowledgements
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Conflict of Interest
The authors declare no competing financial interest.

> IL130. Invited Lecture
Symposium PDT-8 Excitations in PDT (Celine Frochot)

INVESTIGATING THE ULTRASOUND EFFECTS OF DIFFERENT CHEMICAL COMPOUNDS TO HIGHLIGHT THE IN VITRO SONODYNAMIC PROCESS

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Introduction
Ultrasound (US) can be used to trigger the cytotoxicity of chemical compounds, known as sonosensitisers, to yield cancer cell death in an approach that has been defined sonodynamic therapy (SDT). Although SDT mechanisms are still a matter of debate between a cavitation-induced i) photo-activation via sonoluminescence or ii) homolytic splitting of water, it is generally accepted that reactive oxygen species (ROS) are the main effector of sonosensitised cell damage (1). Therefore, this work aims to investigate the US-responsiveness of different chemical compounds in an attempt to clarify the mechanisms underpinning the sonodynamic process

Methods
US were used to trigger the cytotoxicity of different chemical compounds at noncytotoxic concentrations *per se,* such as metalloporphyrin, *i.e.* Pd(II) porphyrin, and chemotherapeutic drugs, *i.e.* doxorubicin and paclitaxel. US-mediated ROS generation were analysed *ex cellulo* by EPR spectroscopy and *in vitro* by DCF-DA flow cytometric assay. The US-mediated anticancer activity of Pd(II) porphyrin, doxorubicin and paclitaxel was then evaluated on the human colon cancer, HT-29, the ovarian cancer, A2780, and the breast cancer, MCF-7, cell lines, respectively. Mitochondrial membrane potential, DNA damage, lipid peroxidation, cell cycle and cell death were analysed by flow cytometric assays and gene expression by real-time-RT-PCR

Results
Our results showed, through EPR analysis, that Pd(II) porphyrin and doxorubicin were more efficient in generating ROS under US exposure than paclitaxel with different patterns of ROS production under US exposure for each compound. These findings were also confirmed when noncytotoxic concentrations of Pd(II) porphyrin and doxorubicin, activated by US in HT-29 and A2780 cells, showed a significant intracellular ROS production and a remarkable reduction in cancer cell growth, along with significant mitochondrial membrane potential impairment and an increase in apoptotic and necrotic cells, respect to paclitaxel in MCF-7 cells. These results suggest that the US-responsiveness of the compounds can be related to their photosensitising properties

Discussion
Since Pd(II) porphyrin and doxorubicin, well known photosensitisers, were able to elicit a significant ROS generation yielding cancer cell death when triggered by US compared to paclitaxel, it might be reasonable to assume that the US-mediated sonosensitiser activation can be due to a sort of photo-activation via cavitation-induced sonoluminescence rather than a radical path process via homolytic splitting of water

Conclusion
The results reported herein support the intracellular ROS generation as the main effector in the sonodynamic process and new insight in the underlying mechanism.

Continued

Acknowledgements
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Conflicts of Interest
The authors declare no conflict of interest

References

1. Yang Y, et al. *Curr Pharm Des*. 2019

> IL131. Invited Lecture
Symposium PDT-8 Excitations in PDT (Celine Frochot)

MOLECULAR SYSTEMS WITH TWO-PHOTON EXCITED PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY AND THERANOSTICS.

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Two-photon excitation PDT (TPE-PDT) is an emerging domain that offers several benefits compared to classical PDT. Indeed, two-photon excitation of the photosensitizer increases the spatial resolution of PDT and when performed within the optical therapeutic window (700-950 nm) allows deeper tissue treatment and with limited photodamage to healthy tissues.[1] Moreover, combining a two-photon photosensitizer with an imaging probe will allow an accurate localization of the therapeutic agent to optimize the medical protocol and the monitoring of the treatment at each step.

Our group has developed new amphiphilic photosensitizers absorbing in the near infrared that consist of porphyrins with an extended π-conjugated system.[2,3] They have shown promising potential for TP-TPE based on their high two photon absorption at 910 nm and singlet oxygen generation. To associate to the PDT photosensitizer an imaging probe, new conjugates with Gd(III) complexes for magnetic resonance imaging were developed.[4] Such combination has shown to improve the efficacy of the contrast agent as compared to the classical GdDOTA used in clinics. These new engineered molecular theranostic agents have also shown to be phototoxic using classical one-photon or two-photon excitation in the near infrared, opening new perspective for a fine-tune treatment depending on the size and localization of the tumor.

> IL132. Invited Lecture
Symposium PDT-8 Excitations in PDT (Celine Frochot)

DEVELOPMENT OF CHEMILUMINESCENT PHOTOSENSITIZERS FOR SELF-ACTIVATING PHOTODYNAMIC THERAPY

Authors: Luís Pinto da Silva[1], Ara Núnez-Montenegro[1], Carla Magalhães[1], Paulo Ferreira[1], Diana Duarte[3], José Rodríguez-Borges[2], Nuno Vale[3], Joaquim Esteves da Silva[1]
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Photodynamic therapy (PDT) is a minimally invasive treatment, already in wide clinical use for treating certain types of cancer due to significant advantages over more conventional cancer therapies: fewer side effects, fast healing of healthy tissue and high spatiotemporal precision. [1,2] Unfortunately, the low penetration of UV-visible light into biologic tissues limits this therapy to treating tumors on or just under the skin or on the outer lining of internal organs and cavities. [1,2].

Herein, we developed a single-molecule photosensitizer capable of intracellular self-activation and with potential tumor-selectivity due to a chemiluminescent reaction involving only a cancer marker [3]. Thus, the photosensitizer can generate a cytotoxic effect without requiring a light source or any added catalyst/co-factor.

Luminescent assays demonstrate both the formation of chemically-induced excited states and the resulting production of cytotoxic singlet oxygen. More importantly, *in vitro* cytotoxicity assays involving the photosensitizer (0.1-100 microM) show significant toxicity for tumor cell lines (breast and prostate cancer), while not inducing toxicity toward normal cells. Furthermore, the novel photosensitizer showed higher cytotoxicity toward tumor cells than that induced by reference drugs (Metformin and Tamoxifen).

In conclusion, this work provides a proof-of-concept for a novel type of photosensitizer that eliminates the current restrictions that photodynamic therapy presents regarding tumor size and localization.

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References

[1] Magalhães, C.M.; Esteves da Silva, J.C.G.; Pinto da Silva, L. *ChemPhysChem* **2016**, *4*, 2286-2294;
[2] Fan, W.; Huang, P.; Chen, X. *Chem. Soc. Rev.* **2016**, *45*, 6488-6519.
[3] Patent PPP59213 (Pending).

> IL133. Invited Lecture
Symposium PDT-8 Excitations in PDT (Celine Frochot)

TISSUE-DEPTH INDEPENDENT LIGHT-BASED CANCER THERAPY

Authors: Samuel Achilefu[1], Rui Tang[1], Gail Sudlow[1], Christopher Egbulefu[1], Alexander Zheleznyak[1], Partha Karmakar[1], Julie Prior[1], Nalini Kotagiri[1]
Presenting Author: Samuel Achilefu
1) Washington University School of Medicine

Light-based therapeutic interventions such as photodynamic therapy (PDT) are currently used in the clinic for treating various human diseases. The exciting combination of light and light-sensitive drugs (photosensitizers, PS) offers a high degree of control to optimize therapy. Despite the promise of PDT, the shallow penetration of light in tissue confines its use to lesions that are accessible to external light source. Furthermore, the reliance on molecular oxygen to generate reactive oxygen species implies that PDT is less effective in hypoxic conditions, which characterize most solid tumours. To overcome these limitations, we developed a treatment paradigm that harnesses the ability of some radiopharmaceuticals to stimulate the production of reactive oxygen species (ROS) from nanophotosensitizers. Unlike conventional photosensitizers, nanophotosensitizers are capable of generating ROS from a variety of oxygen sources, a catalytic process that allows continuous production of cytotoxic ROS for cancer therapy. We have demonstrated the potential to eradicate or inhibit the growth of certain types of solid tumours.[1] Recent results further show the applicability of this treatment method to disseminated and metastatic tumours in mouse models of multiple myeloma and breast cancer.[2] A combination of radionuclide stimulation, Cerenkov radiation, and ROS generation from nanophotosensitizers synergistically overcomes the tissue depth limitation of the current external light delivery methods. The use of radiopharmaceuticals and drugs with a history of human application points to a seamless clinical translation of the new method in future.

References

1. Kotagiri N, Sudlow GP, Akers WJ, and Achilefu S, Nat Nanotechnol. 2015, 10(4):370-379.
2. Kotagiri N, Cooper ML, Rettig M, Egbulefu C, Prior J, Cui G, Karmakar P, Zhou M, Yang X, Sudlow G, Marsala L, Chanswangphuwana C, Lu L, Habimana-Griffin L, Shokeen M, Xu X, Weilbaecher K, Tomasson M, Lanza G, DiPersio JF, and Achilefu S, Nat Commun. 2018, 9(1):275.

> IL134. Invited Lecture
Symposium PDT-8 Excitations in PDT (Celine Frochot)

PHOTODYNAMIC THERAPY BEACON: FROM SMALL MOLECULE TO NANOPARTICLE

Authors: Juan Chen[1], Gang Zheng[1,2]
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1) University Health Network 2) University of Toronto

Background

Photodynamic therapy (PDT) is a clinical treatment in which a photosensitizer (PS) is combined with light and molecular oxygen to generate cytotoxic singlet oxygen. It provides additional tissue selectivity versus conventional chemotherapy in that singlet oxygen is generated only in areas with both accumulated PS and simultaneous illumination by a light source. However, the PS\'s poor *in vivo* behaviors on solubility, bioavailability, tumour accumulation and skin phototoxicity, etc., limited PDT wide application.

PDT beacons development

To improve PDT implementation, we have developed PDT beacon concept by several advanced strategies. (1) molecular PDT beacon: A small molecular PDT agent consists of a PS and a quencher linked by a bio-responsive linker (e.g., peptides or oligonucleotides), which allows the PS's phototoxicity to be silenced until the specific linker-bimolecular target interaction occurring (e.g., protease-mediated linker cleavage or nucleic acid hybridization-induced linker opening). Therefore, PDT can achieve a high level of selectivity by destroying only the targeted cancer cells, while leaving healthy cells unharmed. (2) Nanostructure-driven PS phototoxicity silencing and activation. A porphyrin-HDL has been developed by integrating porphyrin-moiety in HDL-like nanostructure, resulting in a stable ultra-small porphyrin nanoplatform (< 30nm) with excellent biocompatibility and long circulation half-life for efficient and targeted PS delivery. Its prompt tumor intracellular trafficking allows for rapid nanostructure dissociation upon tumor accumulation to release monomeric porphyrins to generate efficiently fluorescence and PDT reactivity that are highly-silenced in intact particles, thus providing an activatable mechanism for low-background fluorescence imaging and tumor-selective PDT. 3) The "porphysome" discovery to extend PS's theranostic capability. Self-assembled by simple porphyrin building blocks, porphysomes enable a "super" absorption and efficient conversion of light energy to heat, making them ideal for photothermal therapy and photoacoustic imaging. Upon nanostructure dissociation, fluorescence and PDT activity of porphyrin monomers are restored, allowing low background fluorescence imaging and activatable PDT. In addition, metal ions can be directly incorporated into the porphyrin building blocks of the preformed porphysomes, unlocking their capability for PET and MRI applications.

Conclusion

PDT beacon designs from small molecule to nanoparticle enable improving PDT therapeutic efficacy, selectivity and safety. Porphysome discovery further extends PS imaging and theranostic multimodality and potentiates synergistic application of multiple therapies, thus representing a new frontier in cancer imaging-guided phototherapy.

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No potential conflict of interest is disclosed

> IL135. Invited Lecture
Symposium PDT-8 Excitations in PDT (Celine Frochot)

PHOTODYNAMIC THERAPY FOR ACTINIC KERATOSIS ON THE FOREHEAD AND SCALP: A RANDOMIZED, CONTROLLED, CLINICAL STUDY EVALUATING A LIGHT EMITTING FABRIC (LEF)

Authors: Serge Mordon[1], Anne Sophie Vignion-Dewalle[1], Henry Abi-Rached[1,2], Elise Thecua[1], Fabienne Lecomte[1], Claire Vicentini[1,2], Pascal Deleporte[1], Ralf-Markus Szeimies[3], Laurent Mortier[1,2]
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Photodynamic therapy (PDT) is an effective conservative treatment for actinic keratoses (AK). However, pain and heterogeneous illumination from rigid panels impede the treatment. To provide a more homogeneous illumination, we have developed a Light-Emitting Fabric (LEF), called Phosistos®.

A randomized, controlled, multicentre, intra-individual clinical study was conducted to compare this new device (P-PDT) to the conventional PDT using a LED Panel (C-PDT). Forty-six patients with grade I-II actinic keratosis of the forehead and scalp were treated with P-PDT on one area (n=280 actinic keratosis) and with C-PDT on the contralateral area (n=280 actinic keratosis). The primary endpoint was the lesion complete response rate at three months with an absolute non-inferiority margin of -10%. Secondary endpoints included patient-reported pain scores, emergence of new actinic keratosis, incidence of adverse events and cosmetic outcome.

Results show that at 3 month follow up, the lesion complete response rate with P-PDT was non-inferior to that with C-PDT (79.3% vs. 80.7%). Moreover, the patient-reported pain score was significantly lower with P-PDT compared to C-PDT (mean ± standard deviation: 0.3 ± 0.6 vs. 7.4 ± 2.3; $p < 0.0001$). At six months, the lesion complete response rate with P-PDT was non-inferior to that with C-PDT (94.2% vs. 94.9.7%). There was a lower incidence of new actinic keratosis for the area treated by P-PDT compared to C-PDT (8.6% of patients vs. 39.1%).

In conclusion, PDT with the innovative LEF device is similar in terms of efficacy than C-PDT in treating actinic keratosis of the forehead and scalp while leading to much lower pain scores and fewer adverse events (**ClinicalTrials.gov Identifier: NCT0307689**).

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Reference

Mordon S, Vignion-Dewalle AS, Abi-Rached H, Thecua E, Lecomte F, Vicentini C, Deleporte P, Béhal H, Kerob D, Hommel T, Duhamel A, Szeimies RM, Mortier L. The conventional protocol versus a protocol including illumination with a fabric-based biophotonic device (the Phosistos protocol) in photodynamic therapy for actinic keratosis: a randomized, controlled, non-inferiority clinical study. Br J Dermatol. 2019 Apr 25. doi: 10.1111/bjd.18048.

> OC048. Oral Communication
Symposium PDT-8 Excitations in PDT (Celine Frochot)

X-RAY INDUCED PDT WITH SCINTILLATING NANOPARTICLES

Authors: Philippe Arnoux[1], Joël Daouk[2], Denise Bechet[2], Alain Delconte[2], Hervé Schohn[2], François Lux[3], Olivier Tillement[3], Christophe Dujardin[3], Benoît Habermeyer[4], Céline Frochot[1], Muriel Barberi-Heyob[2]
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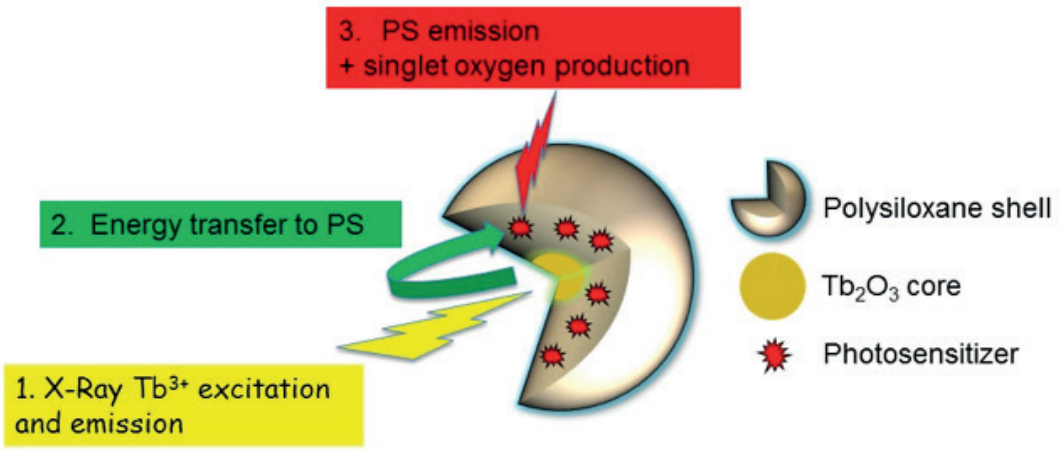
Photodynamic therapy (PDT) is a cancer therapy modality. After excitation of a photoactivable molecule (called photosensitizer) and its energy transfer to oxygen, reactive oxygen species are produced leading to photooxidation reactions. New improvements in the field of PDT concern the development of nanoparticles as delivery carriers for anticancer agents [1, 2].

One of the limit of PDT is the low penetration of light into the tissue. Multiple nanotechnology-based drug delivery systems have been developed to overcome this issue. Our aim is to overcome the constraints imposed by photodynamic therapy (PDT) to treat deep tumor. Recent publications and our results lead us to suggest the development of nanoparticles excited by radiotherapy (standard X-ray from an external corporal energy source) for the treatment of malignant cerebral gliomas [3, 4, 5].

Novel hybrid system of scintillating nanoparticles (nanoscintillators) and PDT photosensitizers enabling excitation of the constructed nanodevices using X-rays, which can penetrate deeply into tissues. Upon exposure to ionizing X-ray radiation, nanoscintillators transfer its energy to the photosensitizers that will activate them. With this novel therapeutic approach, limited light penetration problem could be overcome and activation of the photosensitizer within tumors could be performed using ionizing X-ray radiation. This new modality could allow treatment of deep tumors using lower X-ray radiation dose than conventional radiotherapy [6].



> P088. Poster

Symposium PDT-8 Excitations in PDT (Celine Frochot)

TWO-PHOTON PDT AS A NEW AND MINIMALLY-INVASIVE APPROACH FOR TREATMENT OF MELANOMA CANCER: SYNTHESIS OF PHOTOSENSITISERS

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Presenting Author: Alexis Gosset
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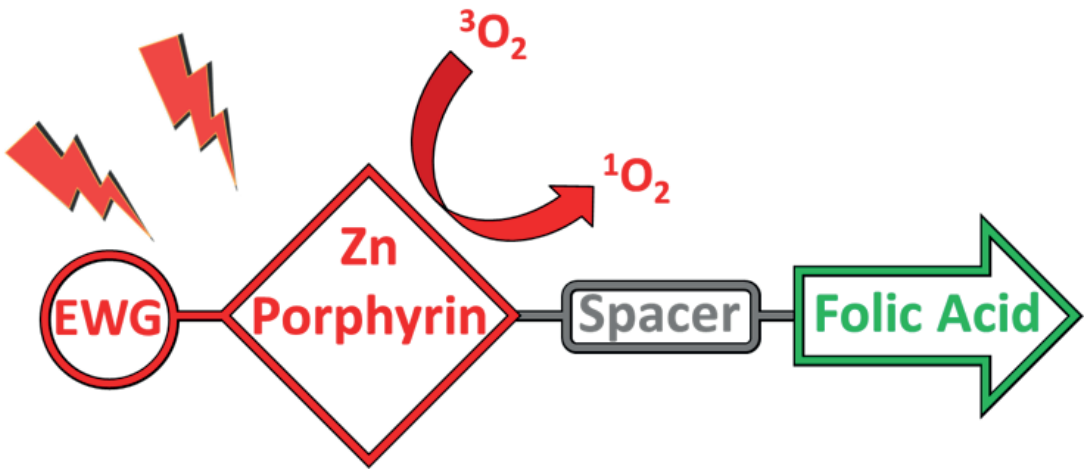
Melanoma is an aggressive cancer with a high rate of mortality and morbidity due to its unresponsiveness to conventional radiotherapy and chemotherapy. Photodynamic Therapy (PDT) is a minimally-invasive technique that combines a photosensitiser, light and oxygen to induce cell death. Although an established modality for non-melanoma skin cancers, it is currently not suitable for melanoma since the (visible) activating light has very limited penetration due to the high tumour pigmentation. This limitation can be overcome by using an innovative strategy based on two-photon excitation photodynamic therapy (TPE-PDT) for the destruction of melanoma.[1] It requires the design of specific two-photon absorbing photosensitisers that bypass the absorption of the high melanin content of melanomas and the use of excitation in the near-infrared (NIR) for a deep penetration of light, to perform an efficient treatment.

Porphyrin derivatives were developed in the group as TP photosensitisers to perform PDT in the NIR.[2] They have very appealing features for TP-PDT, a large TP absorption cross-section (s2 > 1000 GM) in the NIR and significant singlet oxygen production (0.5-0.6 in DMSO). TP excitation at 910 nm performed on cancer cells incubated with such molecules led to important cell death.[3] Based on these results, a new family of PSs with a large π-delocalized system and using folic acid as a vector was designed within sight of treating melanoma cancer.

References

[1] Jenni, S.; Bolze, F.; Sour, A.; Heitz, V. *Chem. Commun.* **2017**, *53*, 12857.
[2] Alam, M. M.; Bolze, F.; Daniel, C.; Flamigni, L.; Gourlaouen, C.; Heitz, V.; Jenni, S.; Schmitt, J.; Sour, A.; Ventura, B. *Phys. Chem. Chem. Phys*. **2016**, *18*, 21954.
[3] Schmitt, J.; Heitz, V.; Sour, A.; Bolze, F.; Ftouni, H.; Nicoud, J.-F.; Flamigni, L.; Ventura, B. *Angew. Chem. Int. Ed.* **2015**, *54*, 169. Schmitt, J.; Heitz, V.; Sour, A.; Bolze, F.; Kessler, P.; Flamigni, L.; Ventura, B.; Bonnet, C.S.; Toth, E. *Chem. Eur. J.* **2016**, *22*, 2775.



> P089. Poster

Symposium PDT-8 Excitations in PDT (Celine Frochot)

DESIGN AND SYNTHESIS OF NEW PHOTOSENSITIZERS WITH OPTIMIZED PHOTOPHYSICAL PROPERTIES FOR APPLICATION IN PHOTODYNAMIC THERAPY

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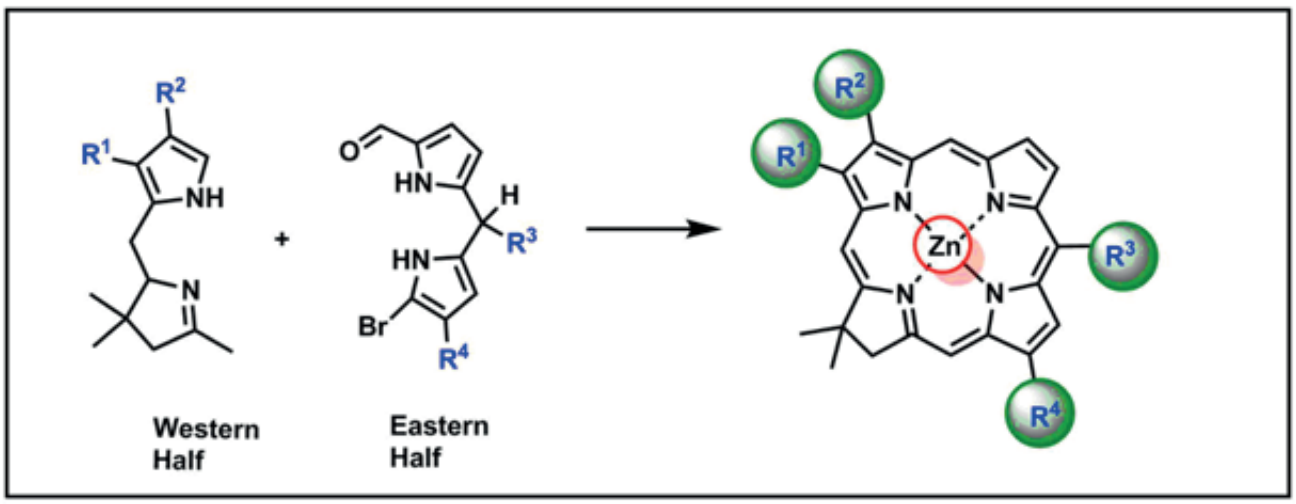
Photodynamic therapy (PDT) is an emerging non-invasive targeted therapy which involves systemic or topical administration of a drug, photosensitizer (PS), which under the effect of a specific wavelength of light and co-existing molecular oxygen, can generate highly reactive singlet oxygen ($^1O_2$) and other reactive oxygen species (ROS). This therapy can lead to specific apoptotic or necrotic cell deaths of the cancer cells.[1] In this study we aim to develop novel non-toxic PSs *i.e.*, chlorins, with potential uses as anticancer or antimicrobial agents.

The current work describes the development of a general synthesis of chlorin photosensitizers bearing various substituents and functional groups, using a method reported by Lindsey and co-workers.[2] A 2+2 condensation of different derivatives of 1-formyl-9-bromo-dipyrromethane (Eastern half) and 2,3,4,5-tetrahydro-1,3,3-trimethyldipyrrin (Western half) yields chlorins with functional moieties in the meso- and *β*-positions, which can be elaborated further (Scheme 1). Optimization of their photophysical properties (red shifted absorption, high triplet state yields, long triplet lifetimes and high singlet oxygen quantum yields) can be achieved through peripheral and conformational modulation. The chlorin periphery is modified *via* Pd(II) catalysed cross-coupling reactions, resulting, e.g., in π-extended chlorins.

References

[1] A. P. Castano, T. N. Demidova, M. R. Hamblin, *Photodiagn. Photodyn. Ther.*, **2004**, *1, 4*, 279–293.
[2] (a) M. Ptaszek, B. E. McDowell, M. Taniguchi, H. Kim, J. S. Lindsey, *Tetrahedron*, **2007**, *63, 18,* 3826–3839; (b) O. Mass, M. Ptaszek, M. Taniguchi, J. R. Diers, H. L. Kee, D. F. Bocian, D. Holten, J. S. Lindsey, *J. Org. Chem*. **2009**, *74, 15*, 5276–5289.



> P090. Poster

Symposium PDT-8 Excitations in PDT (Celine Frochot)

SHORT-WAVE INFRARED FLUORESCENT POLYMERIC NANOPARTICLES FOR IMAGING GUIDED PHOTODYNAMIC THERAPY

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In photodynamic therapy (PDT), fluorescence imaging can be employed to evaluate biodistribution of the fluorescent photosensitizer (PS) to evaluate and visualize its intratumoral accumulation before application of light. However, excitation of the PS while assessing biodistribution results in its premature photobleaching and can cause toxicity to healthy tissues. Use of the additional fluorescent moiety, which is combined with the PS moiety (i.e., conjugated or co-loaded with PS into a delivery vehicle) and can be excited apart of PS activation, can allow for safe, fluorescence imaging-based assessment of PS biodistribution. Combination of PS with infrared fluorescent dyes (IRFD), which absorb light at longer wavelength than the conventional PS and emit in near-infrared (NIR) window of optical transparency for biological tissues has been shown to offer safe monitoring of PS delivery to cancer site, enhanced tumor imaging and PDT.[1,2] In addition to the conventional NIR window (~700-1000 nm), other optical windows have recently been identified in short-wave infrared (SWIR) region (~1000-1700 nm). The reduced tissue scattering and autofluorescence in SWIR spectral region results in a possibility to achieve optical imaging of deeper tissues with better resolution. Thus, use of IRFD-PS formulation that includes IRFD emitting in SWIR range can be beneficial for fluorescence imaging guided PDT.

We developed polymeric nanoparticles (NPs) with polysterene core and thermoresponsive shell of co-polymer of N-isopropylacrylamide and acrylamide, [poly(NIPAM-co-AA)] and loaded them, hydrophobic PS [2-(1-Hexyloxyethyl)-2-devinyl pyropheophorbide-a, HPPH] and IRFD with fluorescence in NIR and SWIR for fluorescence imagine-guided PDT. We have found that the use of SWIR emitting dyes and SWIR fluorescence imaging not only results in higher contrast, sensitivity and penetration depth in comparison with conventional NIR fluorescence imaging. Furthermore, shift of the IRFD absorption to longer wavelengths was found to reduces efficiency of the undesirable electronic excitation energy transfer between PS and IRFD, which negatively affects the efficiency of PDT with PS-IRFD combination. In conclusion, core-shell design of the nanoparticles along with the application of SWIR fluorescent dyes, allowed us to obtain PS nanoformulation that is promising for enhanced PDT guided with advanced optical (SWIR fluorescence) imaging.

References

1. James, N.S.; Joshi, P.; Ohulchanskyy, T. Y., et al. Photosensitizer (PS)-cyanine dye (CD) conjugates: Impact of the linkers joining the PS and CD moieties and their orientation in tumor-uptake and photodynamic therapy (PDT), European Journal of Medicinal Chemistry (2016), 122, 770-785
2. Gupta, A.; Wang, S.; Pera, P.; et al. Multifunctional nanoplatforms for fluorescence imaging and photodynamic therapy developed by post-loading photosensitizer and fluorophore to polyacrylamide nanoparticles. Nanomedicine: NBM (2012) 8 (6), 941–950

> P091. Poster

Symposium PDT-8 Excitations in PDT (Celine Frochot)

LONG-WAVELENGTH ABSORBING RU(II) POLYPYRIDYL COMPLEXES AS PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

Authors: Johannes Karges[Chimi], Gilles Gasser
Presenting Author: Johannes Karges
1) Chimie ParisTech, PSL University, CNRS, Institute of Chemistry for Life and Health Sciences, Laboratory for Inorganic Chemical Biology, France.

During the last decades, cancer has emerged as one of the deadliest diseases worldwide. Photodynamic Therapy (PDT) has expanded the range of treatment opportunities for various types of cancer. In PDT, a preferably non-toxic photosensitiser (PS) is activated at a specific wavelength to generate reactive oxygen species (ROS). As these are highly reactive, they can rapidly interact with essential biomolecules present in cells to trigger their death. The first clinically approved PS was Photofrin®, which is used to treat various types of cancers (e.g. non-small lung, bladder, oesophageal or brain cancer). As the majority of clinically accepted and investigated PSs are based on the same structural scaffold, these compounds are usually associated with similar drawbacks including poor water solubility, tedious synthesis and purification, absorption in the spectral range of the biological environment (i.e. skin, fat, blood), photodegradation and slow clearance from the body causing photosensitivity. To overcome these limitations, there is a need for modification of existing PSs or the development of new classes of PSs. As an emerging class of compounds, Ru(II) polypyridyl complexes have gained much attention due to their attractive chemical and photophysical properties (e.g., high water solubility, high ROS production, chemical stability and photostability). Despite recent research efforts, the majority of investigated Ru(II) polypyridyl complexes lack absorption in the biological spectral window (600-900 nm). During this conference, we will present our results on the synthesis, photophysical and biological evaluation of novel Ru(II) polypyridyl complexes as long wavelength absorbing PSs for PDT.

References

[1] Heinemann, F.; Karges, J.; Gasser, G., Critical Overview of the Use of Ru (II) Polypyridyl Complexes as Photosensitizers in One-Photon and Two-Photon Photodynamic Therapy. *Acc. Chem. Res.* **2017,** *50* (11), 2727-2736.
[2] Jakubaszek, M.; Goud, B.; Ferrari, S.; Gasser, G., Mechanisms of action of Ru (II) polypyridyl complexes in living cells upon light irradiation. *Chem. Commun.* **2018,** *54*, 13040-13059.
[3] Monro, S.; Colón, K. L.; Yin, H.; Roque III, J.; Konda, P.; Gujar, S.; Thummel, R. P.; Lilge, L.; Cameron, C. G.; McFarland, S. A., Transition Metal Complexes and Photodynamic Therapy from a Tumor-Centered Approach: Challenges, Opportunities, and Highlights from the Development of TLD1433. *Chem. Rev.* **2019,** *119*, 797-828.
[4] Karges, J.; Heinemann, F.; Maschietto, F.; Patra, M.; Blacque, O.; Ciofini, I.; Spingler, B.; Gasser, G., A Ru(II) Polypyridyl Complex Bearing Aldehyde Functions as a Versatile Synthetic Precursor for Long-Wavelength Absorbing Photodynamic Therapy Photosensitizers. *Biorg. Med. Chem.* **2019**, 10.1016/j.bmc.2019.05.011.

> IL136. Invited Lecture
Symposium PDT-9 PDT for tissue photobonding and wound closure (Emilio Alarcon)

HYBRID HYDROGELS: AN ANSWER FOR SKIN TISSUE REGENERATION

Authors: Manuel Ahumada[1,2,3], Walfre Franco[2,3]
Presenting Author: Manuel Ahumada
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Patients with full-thickness skin injuries such as burns or chronic wounds must undergo surgical procedures with limited remodeling success that ultimately results in scarring, loss of functionality, and poor cosmetic outcomes. A new surgical procedure that uses microscopic skin columns as grafts has overcome scarring at the donor site; however, their placement into the wound is random because their manipulation is difficult. Soft materials have shown promising results as scaffolds where cells thrive in *in vitro* studies; nonetheless, their application to *in vivo* models remains a challenge. Among other, major limitations are their reduced stiffness and inability to retain water. The objective of the present work is to develop a hybrid matrix with improved mechanical stiffness, reduced water loss, and appropriate biocompatibility for carrying full-thickness skin micrografts into full-thickness skin wounds.

Several single soft materials were mechanically tested to overcome the actual limitations, but none were able to respond to the requirements. Thus, a multi-layer approach was followed to establish a final product denominated hybrid hydrogel. The first two layers of the matrix were developed by combining an elastomer photo-chemically crosslinked to an alginate/acrylamide-based hydrogel with UV light. Full-thickness skin micrografts are incorporated in an additional layer of hydrogel, which was then thermally crosslinked to the first two layers at physiological temperatures to maintain skin viability. Individual and combined layers were physically, chemically, and biologically *in vitro* evaluated.

The multi-layer hybrid material exhibited improved mechanical properties when compared to individual layers and other popular scaffold materials, like collagen and fibrinogen. It also exhibited high retention of the water content after several days, no toxic effects in individual and assembled layers, and cell proliferation from skin micrografts *in vitro*. In summary, we have developed a biocompatible hybrid hydrogel matrix with improved stiffness and water retention, enabling assembly of full-thickness skin micrografts into the matrix as a wound healing patch for *in vivo* applications.

> IL138. Invited Lecture

Symposium PDT-9 PDT for tissue photobonding and wound closure (Emilio Alarcon)

FROM A CONCEPTUAL PROPOSAL TO THE "FUTURE OF WOUND HEALING": THE TRANSLATIONAL PATH OF FRACTIONAL SKIN GRAFTING

Authors: Walfre Franco[1, 2]
Presenting Author: Walfre Franco
1) Wellman Center for Photomedicine, Massachusetts General Hospital 2) Department of Dermatology, Harvard Medical School

The worldwide standard of wound care for full-thickness skin wounds is split-thickness autologous skin grafts, which consists in grafting the upper layers of skin (epidermis and upper dermis) from a healthy donor site onto the wounded site. Clinical outcomes of this procedure are lack of skin function at the wounded site and scarring at both the donor and wounded sites. The adnexal structures that make skin functional are located in the lower dermis. To overcome these limitations we proposed the concept of fractional skin grafting (FSG) that consists in grafting small columns of full-thickness skin. In this talk I will present the development path of FSG which started as a conceptual proposal and has become a medical device for the treatment of skin wounds that will be available for patient care this year. I will discuss the requirements and features that made possible the translation of our technology. I will also discuss many of the barriers that we were able to sort out. In general, these requirements and barriers are not unique to our technology but rather common to the translation of medical devices.

> IL139. Invited Lecture

Symposium PDT-9 PDT for tissue photobonding and wound closure (Emilio Alarcon)

PRECLINICAL STUDIES OF PHOTOCROSSLINKING TECHNOLOGIES FOR TISSUE REPAIR AND REGENERATION

Authors: Robert Redmond[1]
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Introduction

Photosensitized crosslinking (PXL) of proteins in tissue has been investigated for a variety of clinical indications, based on the nature of the clinical problem and the aspect of crosslinking that best addresses the clinical need. Light-activated crosslinking can be used to bond tissues or biomaterials together, to strengthen or stiffen tissues via internal crosslinking for biomechanical purposes, to passivate exuberant inflammatory responses of tissues and in tissue engineering to provide scaffolds that support regenerative medicine applications. Clinical areas that can benefit from these interventions include ophthalmology, peripheral nerve repair, orthopedics, plastic surgery, vascular surgery and GI surgery.

Methods

PXL is performed using a photoactive dye, that is applied to the tissue or tissues involved, and then illuminated with visible light to initiate formation of reactive species that ultimately generate novel crosslinks in structural proteins (e.g. collagen) in the tissue. Most typically the dye used is Rose Bengal, activated with low power green light. For bonding applications, the dye is applied to both tissue surfaces, which are then brought into contact and illuminated for a period of minutes to form a strong photosealed attachment. For all other applications the dye is applied to a tissue surface and then illuminated.

Results and Discussion

Recent pre-clinical studies in vascular graft treatment (passivation and tissue strengthening), peripheral nerve repair (tissue bonding) and penetrating bowel injury (photosealing) will be presented that demonstrate an increased repair and regeneration efficiency of PXL as a primary repair or augmentation to standard surgical repair. In vascular applications PXL stiffens vein grafts to achieve a better compliance match with host artery and reduces endothelial stretch induced injury and cascade to intimal hyperplasia and stenosis. In challenging large gap peripheral nerve repair the advantages of photosealing the neurorrpahy sites with biocompatible nerve wraps improve nerve regeneration in autologous grafts and overcomes some of the limitations of allograft in addressing large gap nerve injuries. Penetrating bowel injuries can be rapidly sealed using by photactivated bonding of strong bio-patches to reduce the risk of bowel leak and resultant sepsis that can result in high morbidity and even mortality.

Conclusions

The PXL platform represents a versatile alternative and/or augmentation to many existing surgical techniques. The late-stage translational studies described here have paved the way for upcoming human studies and adoption into the clinical realm.

Acknowledgements

The excellent contributions of all postdoctoral Fellows and collaborators are gratefully acknowledged, particularly Irene E. Kochevar and Mark A. Randolph at MGH. Generous funding from various branches of the US DoD has made most of these studies possible.

Conflicts of Interest

None

> IL140. Invited Lecture
Symposium PDT-9 PDT for tissue photobonding and wound closure (Emilio Alarcon)

PHOTOCROSSLINKING AND CORNEAL IMPLANTS

Authors: May Griffith[1], Chaoliang He[2]
Presenting Author: May Griffith
1) Maisonneuve-Rosemont Hospital Research Centre and Dept. of Ophthalmology, University of Montreal 2) Key Laboratory of Polymer Eco-materials Changchun Institute of Applied Chemistry, Chinese Academy of Sciences

Introduction
The aim of our work was to examine the posisbility of using photocrosslinking as an alternative to suturing for the retention of cell-free, collagen-based biosynthetic implants as alternatives to donor human corneas. UV photocrosslinking of human corneas with dextran and riboflavin have been used to stabilize collagen fibrils in corneas of patients with keratoconus. This crosslinking procedure should be extendable for use to crosslinking in collagen-based hydrogels as implants.

Methods
Implants made from recombinant human collagen type III (RHCIII) and RHCIII-2-methacryloyloxyethyl phosphorylcholine (RHCIII-MPC) were exposed to solutions of riboflavin and dextran, prior to crosslinking with UV light according to protocols used in clinical crosslinking [1]. A variant of the clinical crosslinking was also tested where benzoylbenzoic acid substituted dextran was produced and used for making photocrosslinked collagen-dextran hydrogels.

Results and discussion
We showed that the clinical collagen photocrosslinking methods allowed for RHCIII-based hydrogels to be crosslinked into the corneas of excised animal eyes [1]. We also show that dextran-based macrophotoinitiators were able to form hydrogels that showed good biocompatibilty with cells suggesting that in the future, hydrogels that can be crosslinked into the cornea as implants or patches are possible.

Conclusions
Photocrosslinking and photocrosslinkers based on dextran could be used in the development of retention methods of biosynthetic implants and also as hydrogels that can be used as patches for damaged or diseased corneas.

Acknowledgements
Photocrosslinking of implants has been published in Reference 1.

Conflicts of interest
RHCIII based implants have been patented by the Ottawa Hospital Research Inst. and Univ. of Ottawa and licensed. MG has no affiliation nor benefits from the licensing.

References

1. Wand K, Neuhann R, Ullmann A, Plank K, Baumann M, Ritter R, Griffith M, Lohmann CP, Kobuch K (2015) Riboflavin-UVA crosslinking for fixation of biosynthetic corneal collagen implants. Cornea 34: 544-549

> IL137. Invited Lecture
Symposium PDT-9 PDT for tissue photobonding and wound closure (Emilio Alarcon)

LIGHT-ACTIVATED BIOMIMETIC MATRICES FOR TISSUE REPAIR

Authors: Emilio I. Alarcon[1], Christopher McTiernan[1], Erik J. Suuronen[1]
Presenting Author: Emilio I. Alarcon
1) University of Ottawa & University of Ottawa Heart Institute

Light-activated tissue bonding using visible light presents numerous advantages for wound closure over conventional surgical suturing, including reduced inflammation and minimal scarring. However, a significant limitation for using tissue bonding is the micrometric space between the tissues of the wound. Also, the currently available suture-less biomaterials cannot modulate the mechanical properties of the resulting bonding structure. Further, wound healing involves the activation of the immune system, which leads to exacerbated production of remodelling enzymes that in turns degrade naturally occurring based photobonding formulations. Thus, developing new technologies able to fine tune mechanical properties of the repaired tissue, while providing suitable resistance to enzymatic degradation and also promoting endogenous tissue vascularization is a must for further advancing in wound healing. In the present contribution, we will discuss a new generation of light-activated peptide-based bio-glues composed by vinyl-modified integrin-specific peptides that are crosslinked using rose Bengal and green light. Interestingly, we have found that the mechanical properties of the resulting materials can be tuned and they present a much higher resistance to enzymatic degradation and suitable pro-vascularization as revealed in murine skin wound models.

Acknowledgements
This work was funded by the Canadian Institutes of Health Research (CIHR) to EIA and EJS. Also the financial contribution Discovery Grant RGPIN-2015-06325 to EIA, the Ontario Ministry of Research Innovation and Science to EIA and University of Ottawa Heart Institute. CM thanks the University of Ottawa Cardiac Endowment Fund.

> OC050. Oral Communication
Symposium PDT-9 PDT for tissue photobonding and wound closure (Emilio Alarcon)

SPLIT FACE STUDY COMPARING CONVENTIONAL MAL PHOTODYNAMIC THERAPY IN MULTIPLE ACTINIC KERATOSIS WITH COMPLETE TIME VERSUS HALF-TIME RED LIGHT LED CONVENTIONAL ILLUMINATION

Authors: Montserrat Fernández Guarino[1], Diego Fernández Nieto[1], Pablo Fonda Pascual[1], Patricia Lizuain Gómez[1], Marta Molins Ruiz[1], Pedro Jaén Olasolo[1]
Presenting Author: Montserrat Fernández Guarino
1) Hospital Universitario Ramón y Cajal. Universidad de Alcalá.

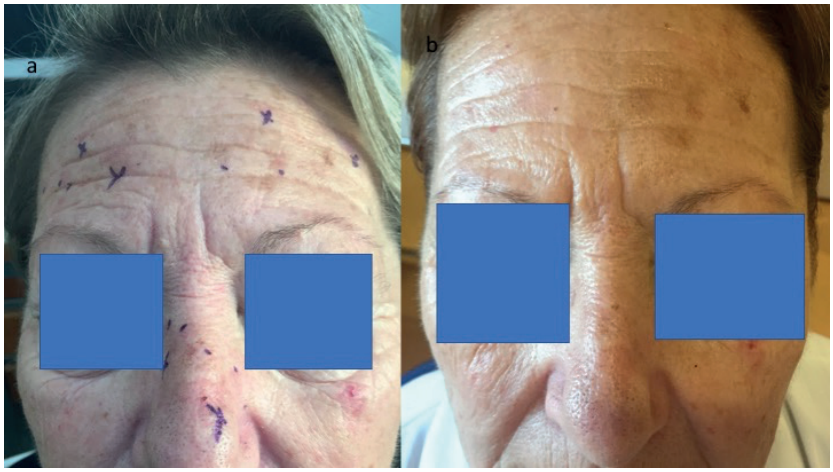
Introduction
Conventional photodynamic therapy (PDT) with methylaminoleavulinic acid (MAL) and daylight PDT have demonstrated similar efficacy in the treatment of actinic keratosis (AK). The reason for the use of daylight is to reduce pain during illumination but daylight has the limitation of the weather conditions. The difference in the doses of red light applied between these two methods suggest that an intermediate dose with red light conventional illumination could be effective in PDT of AK.

Objective
To compare the efficiency of conventional MAL PDT with half time conventional red light illumination in patients with multiple AK.

Material and methods
Adult patients with more than five symmetrically distributed AK were selected. After randomization one area was treated with conventional PDT (AktiliteÒ, 630 nm, 37J/cm^2, 8 minutes), while the contralateral was illuminated half time (AktiliteÒ, 630 nm, 37J/cm^2, 4 minutes). Patients evaluated pain in each different side. Patients were evaluated at baseline, 3 and 6 months after PDT treatment by a blinded dermatologist. A questionnaire to be done at home 24 hours after completing treatment was deliver to the patients to evaluate any side effects.

Results
A total of 774 lesions were treated, 385 with conventional PDT and 389 with half time PDT ($p>0.05$). Conventional PDT was 85% of complete reponse of AK (327/385) at three months and half time PDT was 82% (319/389). At six months, conventional PDT was 70% (268/385) of complete response and half time PDT was 65% (252/389). Pain during illumination was significantly lower in the VAS with the half time protocol with a mean of 5.59 (SD1.48) vs 6.41 (SD1.66) in conventional PDT. No difference in adverse effects were found between protocols.

Conclusion
Conventional PDT with half time illumination in multiple actinic keratosis is as effective as complete time illumination and decreased pain significantly.



> OC049. Oral Communication

Symposium PDT-9 PDT for tissue photobonding and wound closure (Emilio Alarcon)

REMOTELY ACTIVATED BIO-RESPONSIVE PEPTIDE BASED MATRICES FOR SOFT TISSUE REPAIR

Authors: Marcelo Munoz[1], Isabelle Brunette[2], May Griffith[2], Emilio Alarcon[1]
Presenting Author: Marcelo Munoz
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Introduction

Collagen is the main component of the extracellular matrix. This protein had been used extensively in different therapies as a support matrix. Most studies have used animal's extracted collagen, which involves issues of reproducibility, and overall makes clinical translation expensive. Further, most of the matrices assembled to mimic the native extracellular environment are pre-made, with the concept of "cookie cut" being obsolete for fine-tuning the shape and thicknesses of different tissues. In the present work, we have engineered light-sensitive biomimetic matrices using collagen-like peptides [1] anchored to PEG backbones to produce a remotely controllable 3D matrix for in situ tissue repair, independently of the shape and thickness of the organ. We have used non-toxic blue light as an activator of our biomimetic matrix.

Methods

A peptide-PEG based matrix was prepared bearing radical sensitive moieties (acrylate) was prepared using Michael Addition conjugation. The matrix also contained 8-Arm PEG acrylate and MPC. The mixture was optimized for being delivered using a G27 needle. A custom made programable irradiation system was used for crosslinking with a non-toxic light dosage.

Results

We have developed a light-sensitive and biocompatible 3D matrix based on using collagen mimetic assemblies linked to PEG backbones. The matrices have suitable physical and biological properties to allow cell proliferation and biointegration.

Discussion & conclusions

Using light as a remote trigger for in situ matrix assembling of biomimetic structures presents many benefits, including unique temporal and spatial control for the properties of the resulting matrix. In this work, we have developed the first blue-light activated peptide-based polymeric structure for in situ repair of soft tissues. Our cumulative data points towards the importance of encompassing oxygen diffusion and irradiation time in the formation of the matrix.

Acknowledgments

This work was funded by the collaborative research program CHRP (CIHR & NSERC) to EIA, IB, and MG.

References

[1] L.E. O\'leary, J.A. Fallas, E.L. Bakota, et al. (2011) Nat Chem. **3**(10):821-8.

> IL145. Invited Lecture

Symposium PDT-10 PDT for treatment of inflammatory diseases (Qian Peng)

EXTRACORPOREAL PHOTOCHEMOTHERAPY/ PHOTOPOHERESIS

Authors: Robert Knobler[MUW]
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Extracorporeal photochemotherapy is a leukapheresis based therapy that is available in more than 200 centers world wide and was invented by R. Edelson at Columbia University in the 1980s`. During ECP the patients\' whole blood is processed outside the body,white blood cells are collected, exposed to a photoactivateable agent named 8-methoxypsoralen (8-MOP, UVADEX). White blood cells are then exposed to UVA light in a separate chamber and returned to the patient. This is performed on two consecutive days at different intervals depending on the clinical situation. The first and also successful study was performed on patients with cutaneous T-cell lymhpoma and which led to the first FDA approval for this indication in 1988. Additional indications where it has been shown to work are Graft versus Host disease after allogeneic bone marrow transplantation, Chrons disease, Systemic sclerosis, and als rejecton of transplantated organs such as the Lung and the Heart. This is an area of presently increased use.There are a number of open and closed systems in Europe but their efficacy have not been compared in clinical trials. There are no severe side effects associated with this therapy (e.g. grade III-IV WHO). Over 2 million treatments have been performed world wide and the reports are similar. Regarding the mechanisms of action, induction of regulatory T-cells have been shown but the final interpretation is still open for discussion and in the future may turn out to be more disease specific than expected.

> IL141. Invited Lecture
Symposium PDT-10 PDT for treatment of inflammatory diseases (Qian Peng)

MODIFICATION OF EXTRACORPOREAL PHOTOPHERESIS WITH 5-AMINOLEVULINIC ACID

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Extracorporeal photopheresis (ECP), an officially approved modality that exposes isolated white blood cells to photoactivatable 8-methoxypsoralen (8-MOP) and UVA light *ex vivo* followed by returning the treated leukocytes to the body, is used to treat a number of T-cell-mediated disorders including cutaneous T cell lymphoma, graft versus host disease, etc.. However, this modality may kill both diseased and normal cells with little selectivity and clinically it is long-lasting, expensive and only partial response in the majority of treated patients. Furthermore, the mechanism of action is not fully understood, so that it makes difficult to broaden application to additional types of T-cell-mediated diseases. Selective, cheap, short duration and more effective alternatives are thus needed. 5-Aminolevulinic acid (ALA), a precursor of the potent photosensitizer protoporphyrin IX (PpIX), has been shown to selectively induce PpIX in activated T cells and could be an alternative for 8-MOP. The advantages of using ALA for ECP may be selective destruction of proliferative diseased T-cells and production of immunogenic cell death to induce a patient-specific anti-disease immunity.

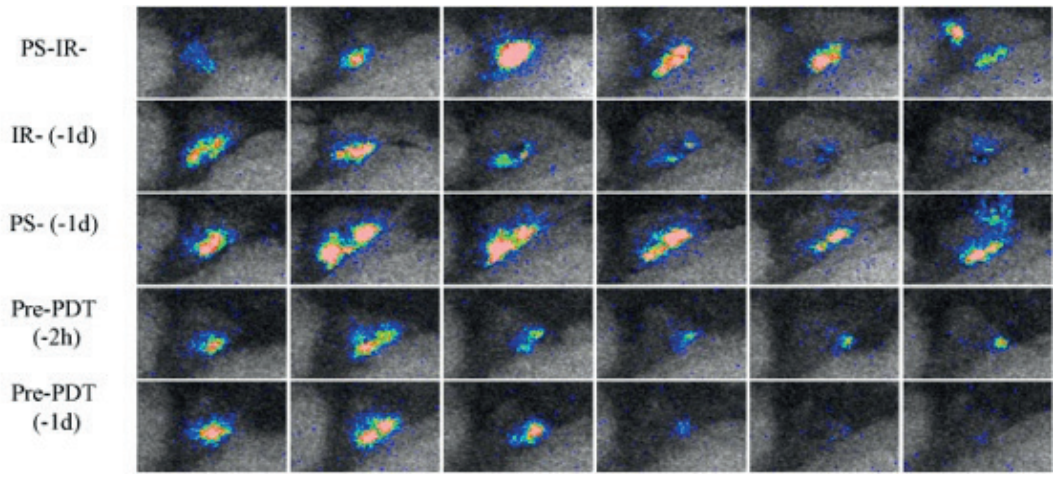
> IL144. Invited Lecture
Symposium PDT-10 PDT for treatment of inflammatory diseases (Qian Peng)

PHOTODYNAMIC THERAPY CAN INDUCE A NON-SPECIFIC PROTECTIVE IMMUNE RESPONSE AGAINST BACTERIAL ARTHRITIS

Authors: Michael R. Hamblin[1949]
Presenting Author: Michael R. Hamblin

1) Massachusetts General Hospital

Photodynamic therapy (PDT) for cancer is known to induce an immune response against the tumor, in addition to its well-known direct cell-killing and vascular destructive effects. PDT is becoming increasingly used as a therapy for localized infections. However there has not to date been a convincing report of an immune response being generated against a microbial pathogen after PDT in an animal model. We studied PDT as a therapy for bacterial arthritis caused by bioluminescent methicillin-resistant *Staphylococcus aureus* infection (MRSA) in the mouse knee. We had previously found that PDT of an infection caused by injection of MRSA (5X10(7) CFU) into the mouse knee followed 3 days later by 1 microg of Photofrin and 635-nm diode laser illumination 5 minutes later using a range of fluences, gave a biphasic dose response in CFU. The greatest reduction of MRSA CFU was seen with a fluence of 20 J/cm^2, whereas lower antibacterial efficacy was observed with fluences that were either lower or higher. We then tested the hypothesis that the host immune response mediated by neutrophils was responsible for most of the beneficial antibacterial effect. We used bioluminescence imaging of luciferase expressing bacteria to follow the progress of the infection in real time. We found similar biphasic results using intra-articular methylene blue (a photosensitizer that was shown to cause least damage to neutrophils in vitro) and red light, and more importantly, that carrying out PDT of the non-infected joint and subsequently injecting bacteria after PDT led to a significant protection from infection. Taken together with substantial data from studies using blocking antibodies we believe that the pre-conditioning PDT regimen recruits and stimulates neutrophils into the soon-to-be infected joint which can then destroy bacteria that are subsequently injected and prevent infection developing. This procedure may be applied prophylactically to patients undergoing high-risk orthopedic surgery.



> IL142. Invited Lecture
Symposium PDT-10 PDT for treatment of inflammatory diseases (Qian Peng)

PHOTODYNAMIC TREATMENT (PDT) OF AN AUTOIMMUNE, INFLAMMATORY DISEASE OF THE ORAL MUCOSA

Authors: Sigrid I. Kvaal[1], Juliane Hesse[1]
Presenting Author: Sigrid I. Kvaal
1) Institute of Clinical Dentistry, Faculty of Dentistry,, University of Oslo

Introduction
Lichen planus is an autoimmune skin disease, which also affects mucous membranes of the oral cavity, oesophagus and genitalia. The erosive form, with recurrent large ulcers, may present in combination with atrophic and reticular mucosa. This painful form of the disease affects eating and drinking habits and have considerable influence on the quality of life The histopathology of oral lesions are characterised by an epithelium of variable thickness and a band of sub-epithelial inflammatory cells mainly T-lymphocytes. There is no known cure for the disease and potent cortisone is prescribed to relieve symptoms(1).

Method
PDT of oral lesions was performed with application of methyl 5-aminovulinate (MAL) [Metvix®] as photo-sensor (PS) on affected mucosa. The area was covered for 15 min and the application repeated after one hour. Three hours after initiation of treatment a biopsy of the affected area was taken and followed by a radiant exposure of 75 J/cm^2 of red light in the region 600 – 660 nm delivered to the affected area at irradiances of 100 – 130 mW/cm^2 using a light-emitting diode (LED) light source(2).

Results
The variable thickness of the epithelium allows easy penetration of active ingredients. Accumulation of porphyrine IX in sub-epithelial inflammatory cells is demonstrated by fluorescence microscopy. Patients experience improvement of the oral condition(2).

Discussion
We have shown the absorption of PS in the inflammatory infiltrate. The treatment showed improvement both of the treated oral mucous membrane and of the non-treated contra lateral side, but no one experienced complete healing. It is possible that local treatment of OLP induces a local tissue reaction of inflammatory cells. Many patients with OLP also have genital affection, but local oral treatment does not have any effect on lichen planus in other sites. This may be because only superficial and regional cells are targeted by the light. Deeper penetration of light and multiple treatment sessions ought to be tested. The type of inflammatory cells which absorbs and converts aminolevulinic acid and the local tissue reaction ought to be investigated further.

Conclusions
MAL-PDT of OLP has shown that inflammatory cells are targeted and this may reduce patient discomfort and pain.

Acknowledgements
Trond Warloe, Qian Peng and Even Angell-Petersen for valuable support, assistance and advice.

Conflict of interest
none

References

1. Gupta S, Jawanda MK. Oral Lichen Planus: An Update on Etiology, Pathogenesis, Clinical Presentation, Diagnosis and Management. Indian J Dermatol. 2015;60(3):222-9.
2. Kvaal SI, Angell-Petersen E, Warloe T. Photodynamic treatment of oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;115(1):62-70.

> IL143. Invited Lecture
Symposium PDT-10 PDT for treatment of inflammatory diseases (Qian Peng)

TUMOR PDT AND STRESS SIGNALING ASSOCIATED INFLAMMATION

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Presenting Author: Mladen Korbelik
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It has become clear that the critical event determining the nature of PDT-induced antitumor response is the engagement of cellular stress signaling networks caused by the infliction of oxidative stress in targeted cancer cells. These signaling networks are responsible for the induction of tumor-localized inflammation as one of the tools for regulating the survival and death in PDT-targeted cancer cells and the means for controlling tissue homeostasis at the treated site. Stress signaling pathways interact with inflammatory and immune pathways at multiple levels. The inflammation is now recognized as a form of innate immune response because it mobilizes multiple humoral and cellular elements of this host defense system including complement, cytokines, neutrophils, mast cells and monocytes/macrophages. Engagement of stress sensor kinases of Integrated Stress Response and Unfolded Protein Response pathways, particularly PERK and IRE1, is closely linked with the activity of NFκB; this transcription factor is one of key regulators of inflammation. Another prominent link is the interaction of IRE1-XBP1 stress signaling axis with Toll-like receptor (TLR) signaling that is vital for production of inflammatory cytokines. On the other hand, the inflamed PDT-treated tumor provides a critical microenvironment for immunogenic cell death (ICD) of cancer cells, their capture and processing by antigen-presenting cells with eventual development of vigorous adaptive immune response raised against targeted lesions.

> OC051. Oral Communication
Symposium PDT-10 PDT for treatment of inflammatory diseases (Qian Peng)

BIOLOGICALLY-ACTIVE PHTHALOCYANINES FOR TARGET-SPECIFIC ANTIMICROBIAL PDT

Authors: Vanya Mantareva[1], Meliha Aliosman[1], Ivan Angelov[1], Yavor Mitrev[1], Vesselin Kussovski[2], Mahmut Durmus[3], Alexander Gisbrecht[4]
Presenting Author: Vanya Mantareva
1) Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria 2) The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences 3) Gebze Technical University, Gebze, Turkey 4) Institute of Electronics, Bulgarian Academy of Sciences, Sofia, Bulgaria

The overuse of antibiotics features as the main reason for a fast development of drug-resistance of wide spreaded life-threaten pathogenic bacteria and fungi associated with human health. The resistance problem has been reinforced the research and development of new therapeutic strategies for effective cure and keeping under control of acute infections. The antimicrobial photodynamic therapy (aPDT) seems very effective method with fast outcome. Among the new generation photoactive drugs with a good perespectives for application to humans are phthalocyanines (Pcs). The study presents new type hybride masromolecules which are consisting of a photoactive phthalocyanines (Pc) substituted with molecules among biologically active substances preferably with cationic charge. These are dual functionality photosensitisers with very promising target-specific action as photoantimicrobials. The presentation summarized our recent expertise in the field of synthesis of novel phthalocyanine complexes and their conjugates with different biologically-active substituents for selective photocytotoxic effect towards pathogens. The photophysicochemical properties of the selected Pcs, such as singlet oxygen and photostability, were evaluated in respect to PDT. *In vitro* PDT results with drug-resistant bacterial species associated with dental health are presented. In conclusion the summary of all collected data underlines the structure-function features of the proposed new phthalocyanines as photoantimicrobials.

Acknowledgements
To the project **KP-06-29/11**, 2018 of the National Science Fund, Bulgaria.

The authors declare no conflict of interest

> P092. Poster

Symposium PDT-10 PDT for treatment of inflammatory diseases (Qian Peng)

TITLE: PHOTOPHYSICAL STUDY OF SN (IV) 5,10,15,20-TETRAKIS(4-BROMOPHENYL) PORPHYRIN AND IN VITRO APPROACH AGAINST LEISHMANIA PHOTOPHYSICAL STUDY OF SN (IV) 5,10,15,20-TETRAKIS(4-BROMOPHENYL) PORPHYRIN AND IN VITRO APPROACH AGAINST LEISHMANIA BRASILIENSIS

Authors: Carlos Díaz[1], Fabián Espitia[1], William Vallejo[1], Doris Gómez[2], Arnold Romero[3]
Presenting Author: Fabián Espitia
1) Universidad del Atlántico 2) universidad de cartagena 3) Universidad Industrial de Santander.

Introduction

Porphyrins have emerged as important sensitizers for Photodynamic therapy (PDT); the porphyrin core and its derivatives have proved high efficacy as both antibacterial and antiviral agents due to its exceptional photodynamic properties; in addition, PDT activity against different biological systems has been reported for porphyrins. According to the WHO, *leishmaniasis* is one of the many neglected diseases. It is estimated that around 350 million people are at risk of contracting it. Currently, there are 12 million people infected, with an annual incidence of 2 million people and close to 30.000 deaths/year. [1,2] In this study, we analyze the photophysical behaviour of 5,10,15,20-tetrakis(4-bromophenyl)-porphyrin (1) and Sn(IV)-porphyrin complex (2)as regards their potential use in PDT against *Leishmania brasiliensis.*

Methods

We synthesized and characterized (1) and (2). Structure compounds were confirmed using ^{1}H-NMR and ^{13}C-NMR, ESI-mass spectrometry, FT-IR spectroscopy, UV-Vis and fluorescence spectrophotometry. Both the singlet oxygen and Fluorescence quantum yield were measured. Finally, Leishmania panamensis (M2903) was used in the in vitro study.

Results and Discussions

Figure 1 shows the UV-Vis spectrum of (1) and (2) in ethyl acetate. The UV-vis spectrum for compound (2) shows one Soret band and only two Q bands. When the Sn (IV) ion coordinates nitrogen atoms inside porphyrin ring, the porphyrin symmetry increases; furthermore, the reduction in the number of Q bands is typical of metalloporphyrin derivative. Under irradiation, the IC_{50} (concentration that inhibited cell growth by 50%) of both compounds was higher compared to the positive control – compound (1) had an IC_{50} of 42.3 μM and (2) had an IC_{50} of 48.2 μM. The activation of sensitizers ensures higher IC_{50} values.

Conclusions

we synthesized and characterized (1) and (2). Results showed that the Sn (IV) ion insertion inside in the porphyrin core reduced significantly Fluorescence Quantum Yield from 0.15 to 0.05. Furthermore, Φ_D increased from 0.55 to 0.59 after metal insertion inside the porphyrin core. The activation of sensitizers ensures higher IC_{50} values. The compounds under study showed high toxicity against parasite under light irradiation. the photophysical and in vitro studies of compounds (1) and (2) suggest that they could be tested as potential sensitizers in photodynamic therapy applications.

References

[1] Alvar J, et. al. 2012, *PLoS One* **7**, e35671
[2] WHO. 2019 Leishmaniasis. *Leishmaniasis*.

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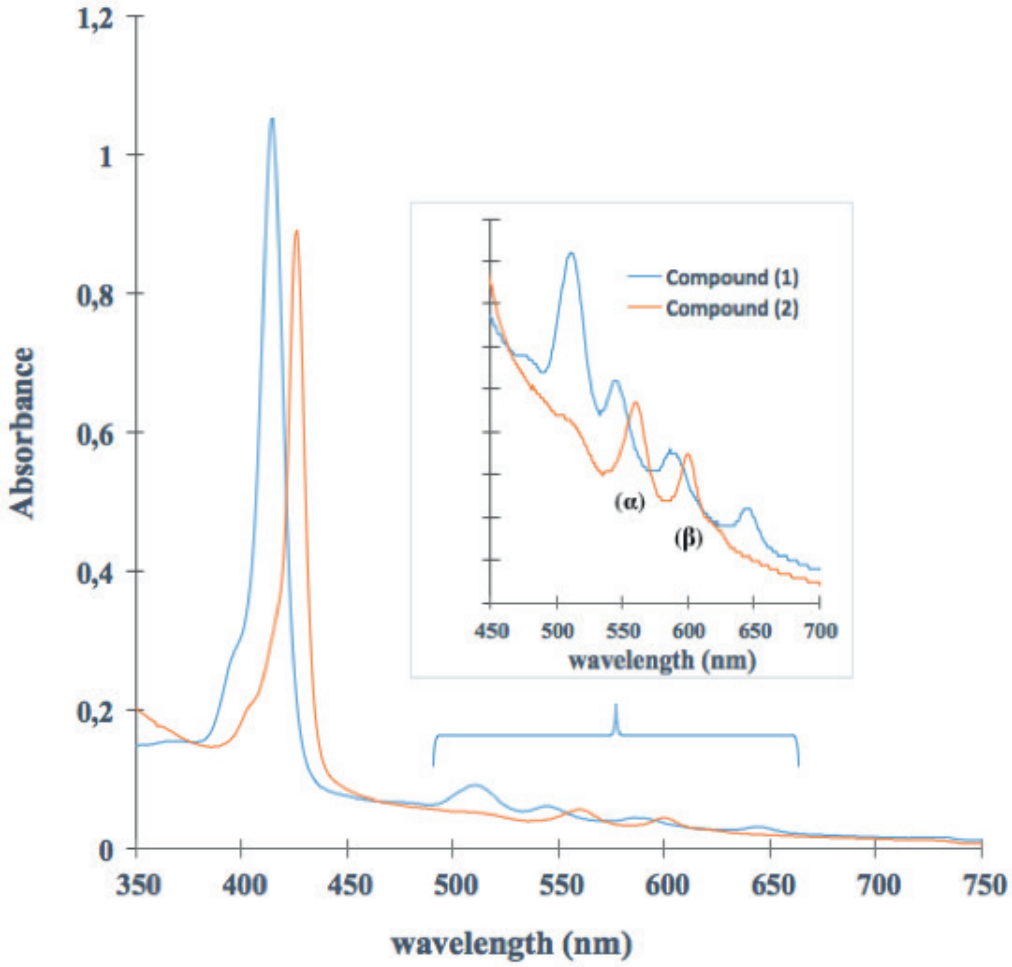
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> OC052. Oral Communication

Symposium PDT-11 Short Communications on antimicrobial PDT (Montserrat Agut)

THE COMBINATION OF SHOCK WAVE THERAPY AND aPDI FOR THE TREATMENT OF WOUND INFECTIONS

Authors: Lisa Karner[1], Magdalena Metzger[1], Heidi Strauß[1], Roland Rose[1], Carina Wagner[1], Heinz Redl[1], Peter Dungel[1]
Presenting Author: Lisa Karner
1) Ludwig Boltzmann Institute for Experimental and Clinical Traumatology

Introduction

The urgent problem of antimicrobial resistances was highlighted in the global action plan by the WHO in 2014. This call for rapid intervention demanded new strategies and therapy approaches before the human society might again face untreatable infections like back in the pre-antibiotic era.[1] Antimicrobial photodynamic inactivation (aPDI) as well as shock wave (SW) therapy are both known as innovative and antibiotic resistance-independent[2] biophysical approaches in the field of wound healing[3] and treatment of wound infections.[4-6] The aim of this study was to investigate the combination of these therapies in order to compensate each other's limitations and strengthen their antibacterial effectiveness.

Methods

Model germs of the genera Escherichia and Staphylococcus, that play an important role in wound infections, were incubated with the photosensitizer (PS) methylene blue (MB) and thereafter exposed to shock wave treatment. These preconditioned bacteria were then exposed to Repuls pulsed red LED light (635 nm, 2.5 Hz, 50 % pulse rate) for aPDI treatment. Electrohydraulic, electromagnetic and radial shock waves were tested for their direct bactericidal activity and their preconditioning effect on aPDI treatment. Bactericidal activity was assessed by analyzing colony forming units (CFU).

Results and Discussion

Independent of the bacterial stain, the mode of shock wave generation and the number of impulses, shock wave treatment did not show any direct bactericidal effects. However, preconditioning with shock waves significantly diminished the effects of subsequent aPDI treatment in all bacterial strains used. Indirect influences like methylene blue degradation and pH changes were ruled out. Time course analyses suggested that the preconditioning effect was only short term. Habituation or selection effects could not be observed.

Conclusion

As shock wave treatment is an emerging and promising therapy and has already been suggested to treat wound infections, e.g. for biofilm disruption[7,8], the knowledge about this diminishing effect of shock waves on aPDI efficiency is of utmost importance for clinical applications. To avoid negative outcomes for patients, the duration of the effect, as well as its mechanisms have to be further studied in order to recommend save application protocols for this combinatorial approach.

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References

1 WHO. Antimicrobial resistance: global report on surveillance, 2014.
2 Kashef, N. & Hamblin, M. R. Drug Resist Updat 31, 31-42, 2017.
3 Weihs, A. M. et al. J Biol Chem 289, 27090-27104, 2014.
4 Biel, M. A. Methods Mol Biol 635, 175-194, 2010.
5 Pereira de Lima Carvalho, D. et al. Lasers Med Sci 29, 113-120, 2014.
6 Kashef, N. et al. Iran J Microbiol 3, 36-41 2011.
7 Gnanadhas, D. P. et al. Sci Rep 5, 17440, 2015.
8 Wanner, S. et al. J Bone Joint Surg Br 93, 824-827, 2011.

> OC053. Oral Communication

Symposium PDT-11 Short Communications on antimicrobial PDT (Montserrat Agut)

FIGHTING STAPHYLOCOCCUS AUREUS RESISTANCE WITH LIGHT: THE BRIGHT FUTURE OF PHOTO-ACTIVATABLE IMMUNOCONJUGATES

Authors: Mafalda Bispo[1], Andrea Anaya Sánchez[1], Marina López Álvarez[1], Marjolein Heuker[1], Elisa Raineri[1], Francisco Romero Pastrana[1], Girbe Buist[1], Marleen van Oosten[1], Gooitzen M. van Dam[2], Jan Maarten van Dijl[1]
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Staphylococcus aureus is an opportunistic pathogen with the ability to develop biofilms that protect it against the host immune system and antimicrobial agents. Moreover, treatment of *S. aureus* infections is increasingly complicated by high-level antibiotic resistance as exemplified by methicillin-resistant *S. aureus* (MRSA). Therefore, there is a pressing need for antimicrobial approaches that inactivate pathogens effectively without the risk of inducing resistances. Antimicrobial photodynamic therapy (aPDT) has been proposed as an alternative approach for the inactivation of bacteria. The *per se* non-toxic photosensitizer (PS) becomes toxic only upon activation with visible light by producing reactive oxygen species (ROS), mostly singlet oxygen ($^1O_2$), that will kill the bacteria[1]. The quest for the ideal aPDT photosensitizer is an intriguing challenge. Fluorescence properties, water solubility, high $^1O_2$ production and specificity to the target disease are important parameters to consider. Bioconjugation of photosensitizers with monoclonal antibodies (mAbs) is a very attractive strategy due to the ability of mAbs to recognize specific antigens, thereby improving the specificity of the drug to the site of injury/disease[2].

Our group developed a human mAb (1D9) that specifically targets the immunodominant staphylococcal antigen A (IsaA) of *S. aureus* - a non-covalently cell wall-attached conserved protein[3]. The potential application of 1D9 as a non-invasive diagnostic tool for fluorescent image-guided surgery and selective debridement of infected tissue was recently demonstrated in murine infection models[4]. Therefore, we conjugated 1D9 with a near-infrared PS and tested its efficacy as an aPDT agent. The production of ROS after aPDT was demonstrated by transmission electron microscopy with the diaminobenzidine photooxidation method. Importantly, the immunoconjugate was able to destroy the *S. aureus* biofilm shell upon red light irradiation, decrease *S. aureus* viability in a post-mortem implant model and protect MRSA-infected *Galleria mellonella* larvae. Altogether, these results show that our approach has a high potential for therapeutic applications in the fight against highly drug-resistant bacteria.

References

1. Cieplik F *et al*. Antimicrobial photodynamic therapy – what we know and what we don't. *Crit Rev Microbiol*. 2018; 44(5):571–89.
2. Bullous AJ *et al*. Photosensitiser-antibody conjugates for photodynamic therapy. *Photochem Photobiol Sci*. 2011; 10(5):721–50.
3. Romero Pastrana *et al*. *et al*. Noninvasive optical and nuclear imaging of Staphylococcus-specific infection with a human monoclonal antibody-based probe. *Virulence*. 2018; 9(1):262-272.
4. Zoller SD *et al*. Multimodal imaging guides surgical management in a preclinical spinal implant infection model. *JCI Insight*. 2019;4(3).

> OC054. Oral Communication

Symposium PDT-11 Short Communications on antimicrobial PDT (Montserrat Agut)

PHOTODYNAMIC INACTIVATION OF CAMPYLOBACTER JEJUNI - AN INNATE SENSITIVITY

Authors: Peter Walker[1], Julia Weinstein[2], David Kelly[1]
Presenting Author: Peter Walker
1) Department of Molecular Biology and Biotechnology, The University of Sheffield, Western Bank, Sheffield. S10 2TN, UK. 2) Department of Chemistry, The University of Sheffield, Western Bank, Sheffield. S10 2TN, UK.

Introduction

Campylobacter jejuni is the leading cause of bacterial foodborne gastroenteritis worldwide, with an estimated 400 million human infections occurring each year through handling of undercooked chicken meat. Although disease is usually self-limiting, with symptoms ranging from mild diarrhoea to acute dysentery, complications can lead to severe neurological disorders such as Guillain-Barré syndrome, a polio-like form of paralysis. As *C. jejuni* is an important foodborne pathogen, its control is of great significance, especially as antibiotic resistant strains are emerging. Photodynamic therapy (PDT) has emerged as an innovative non-antibiotic approach to inactivate *Campylobacter jejuni* by inducing oxidative damage through excitation of endogenous photosensitizer molecules. The presence of oxygen sensitive enzymes coupled with an abundance of light absorbing photosensitizer deems *C. jejuni* much more susceptible to the effects of PDT than other Gram-negative pathogens.

Results

C. jejuni is far more sensitive to killing by 405 nm light than other enteric bacteria with a 6 log reduction seen after a 200 Jcm^2 light dose by exciting only endogenous photosensitisers, naturally synthesised by the bacteria. Whole cell spectroscopy has revealed this 405 nm absorbing chromophore is less abundant in other bacteria, which might partly explain this sensitivity. Using HPLC and mass spectrometry, we have successfully identified this chromophore. Although increasing light doses leads to increased intracellular ROS production, at a bacteriostatic light dose mutants in key oxidative stress defence genes do not show increased killing and known ROS sensitive enzymes are not inactivated. Transcriptome data suggests protein damage rather than oxidative stress may be important in preventing growth at these moderate light doses. Further transcriptomic experiments have revealed the global transcriptomic response at both bacteriostatic and bactericidal light doses within the same time course.

Conclusion

Our results have revealed an unexpected complexity in the way visible light interacts with *C. jejuni* and that ROS may not be the sole cause of damage. Global transcriptomic analysis has shown for the first time the cellular response to endogenously generated ROS. The results from these experiments highlight the innate sensitivity of *C. jejuni* to light induced damage and lay the foundations for the use of this technology in reducing the bacterial load on shop bought chicken carcases.

> OC055. Oral Communication

Symposium PDT-11 Short Communications on antimicrobial PDT (Montserrat Agut)

ANTIMICROBIAL SINGLET OXYGEN PHOTOSENSITIZERS BASED ON GLYCODENDRIMERIC [60]FULLERENE DERIVATIVES

Authors: David García Fresnadillo[Depar], Antonio José Sánchez-Arroyo[Depar], Sergio Ramírez Barroso[Depar], David Bernardo[Servi], Alicia C. Marín[Servi], Adrián G. McNicholl[Servi], Javier P. Gisbert[Servi], Tamara Soler[Servi], Claudio Alba[Servi], Teresa Alarcón[Servi], Santi Nonell[Insti], Nazario Martín[Depar]
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Novel photosensitizers based on glycodendrimeric derivatives of [60]fullerene have been developed for *H. pylori* inactivation by antimicrobial photodynamic therapy (aPDT). This Gram (–) bacterium colonizes the gastric mucosa and is responsible for various severe gastric diseases, being considered as one of the most widespread human pathogens. However, antibiotic resistance has reduced the eradication rates of commonly used therapies to less than 80%.[1] Therefore, the search for promising alternative antimicrobial treatments such as aPDT is considered an urgent issue.[2]

[60]fullerene has been derivatized by click chemistry with glycodendrons carrying 6, 12 or 18 L-(–)-fucose units in order to favour multivalent interactions with the bacterium, based on the fact that *H. pylori* expresses membrane proteins (*e.g.*, BabA and SabA adhesins) able to interact with glycoconjugates bearing this terminal sugar during the colonization process of gastric mucosa.[3] Fluorescein-labelled glycodendrons have also been synthesized and the interaction between *H. pylori* and the fucosylated fluoroprobes has been demonstrated by flow cytometry. The photosensitizers and fluoroprobes were structurally characterized, and tendency to aggregation in water was proven by using the pendant drop method. Photophysical characterization was carried out by UV-VIS absorption and emission (steady-state and time-resolved) spectroscopies. *In vitro* photodynamic inactivation tests of *H. pylori* were performed under blue light (LED lamp, λ_{em}^{max} 465 nm, average fluence rate 60.5 mW cm^{-2}) in the presence/absence of the photosensitizers. Flow cytometry and colony counting methods were used to determine *H. pylori* survival after treatment.[4] Singlet oxygen production quantum yields (Φ_Δ) in the 0.02–0.27 range have been determined for the different photosensitizers tested. Aggregation in water plays a significant role in their Φ_Δ values. The [60]fullerene derivatives carrying 12 and 18 fucose units photoinactivate the *H. pylori* population by four orders of magnitude after 30 minutes of blue light irradiation. In summary, several novel [60]fullerene photosensitizers derivatized with different glycodendrons have demonstrated to bind and efficiently photoinactivate *H. pylori* pathogenic bacterium, despite the self-aggregation behaviour of the glycodendrimeric photosensitizers.

Financial support by the European Research Council ERC-320441-Chirallcarbon and the Ministerio de Economía y Competitividad (MINECO) of Spain (projects CTQ 2014-52045-R, CTQ2017-84327-P, CTQ2017-83531-R, CTQ2015-71896-REDT and CTQ2016-78454-C2-1-R) is acknowledged. A.J.S.-A. thanks the Universidad Complutense de Madrid for its predoctoral fellowship (CT4/14).

There are no conflicts to declare.

References

[1] (a) N. R. O'Morain *et al*. *Helicobacter,* **2018**, *23*, e12519; (b) J. P. Gisbert and A. G. McNicholl. *Helicobacter*, **2017**, *22*, e12392.
[2] (a) M. Wainwright, *et al*. *Lancet Infect. Dis.,* **2017**, *17*, e49-e55; (b) C. Simon *et al*. *J. Photochem. Photobiol. B: Biol*., **2014**, *141*, 113-118. (c) M. Calvino-Fernández *et al*. *Eur. J. Med. Chem*., **2013**, *68*, 284-290.
[3] (a) K. Moonens *et al*. *Cell Host Microbe*, **2016**, *19*, 55-66; (b) S. S. Pang *et al*. *J. Biol. Chem.,* **2014**, *289*, 6332-6340.
[4] C. Alba, *et al*. *Helicobacter,* **2018**, *23*, e12525.

> OC056. Oral Communication

Symposium PDT-11 Short Communications on antimicrobial PDT (Montserrat Agut)

EASY PHENALENONE FUNCTIONALIZATIONS FOR ANTIMICROBIAL ACTIVITIES AND DRUG DELIVERY

Authors: Godard Jérémy[1], Bregier Frédérique[1], El Kihel Abdechakour[1], Chaleix Vincent[1], Chapron David[2], Rosilio Véronique[2], Sol Vincent[1]
Presenting Author: Jérémy Godard
1) Laboratoire PEIRENE, EA 7500, Faculté des Sciences et Techniques, Université de Limoges, 123 avenue Albert Thomas, 87060 Limoges, France. 2) Institut Galien Paris-Sud, UMR CNRS 8612, Université Paris-Sud, 5 rue J-B. Clément, 92296 Châtenay-Malabry, France.

Phenalenone is a photosensitizer known for its high singlet oxygen quantum yield (Δ^1O_2) [1] and its remarkable range of solubility that justify its use as a reference for the evaluation of the Δ^1O_2 [2] as well as its application as a photo-antimicrobial agent [3]. Recently, the synthesis of phenalenone became more efficient, with yield dropping from 26% [4] to 90% [5]. Some functionalization are also described but there is an obvious lack of diversity compared to other photosensitizers like porphyrins or phthalocyanines, and most of them impact significantly the Δ^1O_2 [6].

In this work, initial synthesis and functionalization of the phenalenone were optimised. All main functions were fixed with good to excellent yield by reaction of a halogenated derivative of the phenalenone (PNCl) with simple reactants at a multigram scale and ambient temperature (**Figure 1**). More than twenty new phenalenone derivatives were completely described, and although all of them can easily find practical applications, some of them turn out to be very interesting for the surface grafting. So, cellulosic materials were functionalized with phenalenone and their antimicrobial activity was evaluated. On the other hand, vesicles made with fatty acid – phenalenone (PN) derivatives were elaborated and their release capabilities were studied **(Figure 2)**.

This work demonstrates that phenalenone is a very efficient, easy to handle photosensitizer which deserves more attention for biological applications.

References

[1] E. Oliveros, P. Suardi-Murasecco, T. Aminian-Saghafi, A.M. Braun, H.J. Hanseu, *Helvetica Chimica Acta*, **1991**, *74*, 79-90; [2] R. Schmidt, M. Bodesheim, *J. Phys. Chem.*, **1994**, *98*, 2874-2876; [3] A. Späth, C. Leibl, F. Cieplik, K. Lehner, J. Regensburger, K.A. Hiller, W. Bäumler, G. Schmalz, T. Maisch, *J. Med. Chem.*, **2014**, *57*, 5157-5168. [4] L.F. Fieser, E.B. Hershberg, *J. Am. Chem. Soc.*, **1938**, *60*, 1658-1665; [5] O. Anamimoghadam, "9-C-substituted phenalenones as promising precursors for the synthesis of novel stable phenalenyl-type cations and radicals", *PhD diss.*, University of Glasgow, **2013**; [6] C. Sandoval-Altamirano, J.R. De la Fuente, E. Berrios, S.A. Sanchez, N. Pizarro, J. Morales, G. Gunther, *J. Photochem. Photobiol. A : Chem.*, **2018**, *353*, 349-357.

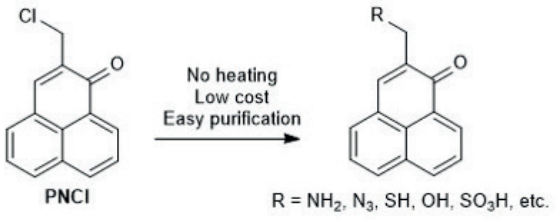

Figure 1. Examples of phenalenone functionalization

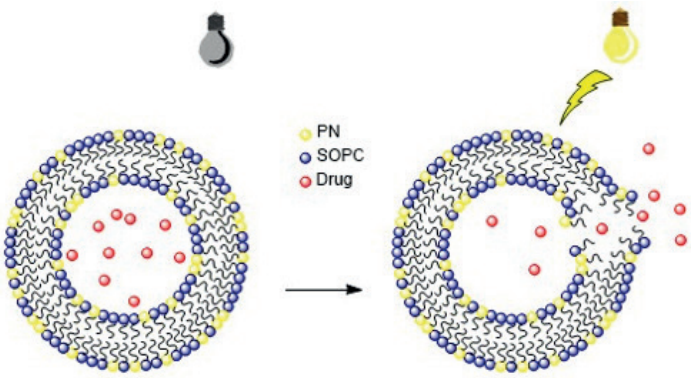

Figure 2. Release capabilities of photoactivable liposome

> OC057. Oral Communication

Symposium PDT-11 Short Communications on antimicrobial PDT (Montserrat Agut)

PHOTODYNAMIC INACTIVATION OF MICROORGANISMS AND PHOTOTRANSFORMATION OF MICROPOLLUTANTS: THE WATER MATRICES ROLE IN THE EFFICIENCY OF THE PROCESS

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Wastewater (WW) containing pathogenic microorganisms (MO), pharmaceuticals and personal care products (PPCPs) and soluble microbial products (SMPs), is subject of concern, affecting the quality of receiving waters. Traditional methods to reduce pathogens concentration by disinfection processes (chlorine, UV) are expensive, unsafe and sometimes ineffective, highlighting the need for new technologies. The promising results of photodynamic inactivation (PDI) of MO with photosensitizers suggests its application not only to MO inactivation, but also to the photodegradation of micropollutants.

One of the aims of our work is to assess photodynamic action applicability for the microbial inactivation and chemical contaminants photodegradation on WW. We have been evaluating the efficacy of PDI on different microorganisms' species, including pathogenic ones, as well as the efficiency of the reactive oxygen species based photochemical treatment in the photodegradation of chemical pollutants. We have been performed experiments with different photosensitizers, different light sources and different water matrices compositions to inquire about some influencing conditions in the effectiveness of both microbial photodynamic inactivation and chemical pollutants photodegradation. Some results of bacterial inactivation in phosphate buffered saline (PBS), distilled water, tap water, well water, river water, swimming pool, wastewater and aquaculture water will be presented and discussed. The phototransformation, using the same PDI protocol, of phenol and of two antibiotics frequently found in the natural waters will be also presented and discussed.

Thanks are due to the University of Aveiro, to FCT/MEC for the financial support to QOPNA Research Unit (FCT UID/QUI/00062/2019) and Centre for Environmental and Marine Studies (CESAM) (UID/AMB/50017/2019), to FCT/MCTES through national funds and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020, and also to the Portuguese NMR Network. Maria Bartolomeu also thanks FCT for her PhD grant (SFRH.BD.121645.2016).

> OC058. Oral Communication
Symposium PDT-11 Short Communications on antimicrobial PDT (Montserrat Agut)

LIGNIN-STABILIZED METAL NANOPARTICLES: PHOTO-INDUCED ANTIBACTERIAL ACTIVITY

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The use of metal nanoparticles as antimicrobial agents is directly related to the type of coating agents utilized to stabilize the particles in the biological media, while retaining the antimicrobial activity.[1] The growing interest in the use of environmentally friendly capping agents, as well as, the use of sustainable resources for antimicrobial applications,[2-3]has led our group towards the use of non-toxic and inexpensive capping agents.[4] The use of natural compounds, that can find value-added application while being eco-friendly materials, is important not only to favour sustainable practices but also to protect the environment. Here we explore the use of lignin, the second most abundant natural polymer on earth after cellulose, as an alternative reducing and capping agent for the synthesis and stabilization of metal nanoparticles for potential applications as antimicrobial agents. Lignin is a natural, heterogeneous and cross-linked phenolic polymer; mainly obtained as a waste product in the wood-pulp and sugar-cane milling industries. Additionally, as reported, lignin is also environmentally compatible, biodegradable and harmless for human health.

Here we present the one-pot thermal and photochemical syntheses –under mild conditions– of lignin-doped silver and gold nanoparticles and their use as antimicrobial agents against *Escherichia coli* and *Staphylococcus aureus*. The nature of the lignin as well as the metal are directly involved in the antimicrobial activity observed in these nanocomposites. It is believed the interaction of the nanocomposites with the bacterial cell wall can be governed by the lignin structure helping not only on the stability of the particles but also on their selectivity towards different type of bacteria. Whereas one of the nanocomposites is innocuous under dark conditions and shows photoinduced activity only against *S. aureus*, the rest of the lignin-coated silver nanoparticles studied show antimicrobial activity under dark and light conditions for both bacteria strains. Additionally, only photoinduced activity is observed for lignin-coated gold nanoparticles. Importantly, the particles are non-cytotoxic towards human cells at the bactericidal concentrations. Preliminary assays show these silver nanoparticles as potential antimicrobial agents towards *S. aureus* biofilm eradication.

References

1. Dragoman, R. M.; Grogg, M.; Bodnarchuk, M. I.; Tiefenboeck, P.; Hilvert, D.; Dirin, D. N.; Kovalenko, M. V. Surface-Engineered Cationic Nanocrystals Stable in Biological Buffers and High Ionic Strength Solutions. *Chem. Mater.* **2017,** *29*, 9416-9428.
2. Tsai, T. T.; Huang, T. H.; Chang, C. J.; Ho, N. Y. J.; Tseng, Y. T.; Chen, C. F. Antibacterial cellulose paper made with silver-coated gold nanoparticles. *Sci. Rep.* **2017,** *7*.
3. Sathiyabama, M.; Manikandan, A. Application of Copper-Chitosan Nanoparticles Stimulate Growth and Induce Resistance in Finger Millet (Eleusine coracana Gaertn.) Plants against Blast Disease. *J. Agric. Food. Chem.* **2018,** *66*, 1784-1790.
4. Weerasekera, H. D.; Silvero, M. J.; da Silva, D. R. C.; Scaiano, J. C. A database on the stability of silver and gold nanostructures for applications in biology and biomolecular sciences. *Biomater. Sci.* **2017,** *5*, 89-97.

> OC059. Oral Communication

Symposium PDT-11 Short Communications on antimicrobial PDT (Montserrat Agut)

ACETYLATED LIGNIN NANOPARTICLES AS A VEHICLE FOR PHOTOSENSITIZERS IN ANTIMICROBIAL PHOTODYNAMIC TREATMENT

Authors: Nidia Maldonado-Carmona[1], Guillaume Marchand[1], Mario J. F. Calvete[2], Mariette M. Pereira[2], Tan-Sothea Ouk[1], Claude Calliste[1], Nicolas Villandier[1], Stèphanie Leroy-Lhez[1]
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Introduction

In the present work, acetylated lignin was evaluated as a producer of reactive oxygen species (ROS), as well as a delivery system for non-hydro soluble photosensitizers (PS) with antimicrobial activity.

Methods

Lignin acetylation was carried out on Kraft-lignin: lignin was dissolved on pyridine and anhydride acetic (v/v, 1:1) at 25 °C under argon atmosphere for 48 h. Lignin and acetylated lignin (AcLi) singlet oxygen and superoxide anion productions were monitored by EPR spectroscopy. AcLi nanoparticles (@AcLi) were prepared as follow: a THF AcLi solution (2 mg/mL) was dialyzed (12-14 KDa cut off) against distilled water for 24 h; then nanoparticles were recovered through centrifugation (8,000 rpm, 1 h). Charged @AcLi were prepared with the addition of 2 mg of zinc (II) 2,9,16,23-tetra(N-imidazolyl)phthalocyanine (TImPcZn) or 5,10,15,20-tetrakis(4-hydroxyphenyl)-21*H*,23*H*-porphine (THPP) at the AcLi starting solution. Antimicrobials tests were done against *Enterococcus faecalis* or S*taphylococcus aureus* planktonic cells, under white LED-light (147.5 J/cm^2).

Results and Discussion

Lignin acetylation enhances singlet oxygen and superoxide anion generations by a factor of *ca.* 6 and 3, respectively, compared with Kraft lignin, after irradiation of white light for 30min[1]. The direct comparison with @AcLi is not possible for solubility reasons. However, @AcLi are shown to be still able to produce ROS and thus can be used as PS. The effect of tetrapyrrolic PS and the PS@AcLi systems on two Gram positive bacterial strains survival is resumed in Table 1. These results indicate that @AcLi work as a vehicle for PS that still have antibacterial activity. However, the efficiency of the PS is diminished, as to eliminate 99.9 % of bacteria, higher concentrations are needed, when compared to the naked PS.

Table 1. Bacterial survival after Antimicrobial Photodynamic Treatment

Sample	Concentration	Bacterial strain	Bacterial survival (%)	
			Dark	Light (147.5 J/cm^2)
TImPcZn	4.85 µM	*E. faecalis*	112.52 ± 34.28	< 0.1
TImPcZn@AcLi	50 µM		107.00 ± 27.05	< 0.1
THPP	40 nM	*S. aureus*	98.52 ± 23.88	0.0094 ± 0.023
THPP@AcLi	320 nM		85.95 ± 12.85	1.61 ± 0.82
@AcLi	1.6 mg/mL	*E. faecalis*	88.79 ± 30.20	115.03 ± 10.11
		S. aureus	108.24 ± 13.39	89.80 ± 22.18

Conclusions

@AcLi work as a vehicle for antimicrobial PS. The current tests were carried on at ideal conditions, thus further testing is needed to investigate the effectiveness of systems studied in non-ideal conditions.

Acknowledgements

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References

1. Marchand, G. *et al., ChemistrySelect* **3**, 5512–5516 (2018).

> OC060. Oral Communication

Symposium PDT-11 Short Communications on antimicrobial PDT (Montserrat Agut)

EXPOSURE TO VISIBLE LIGHT POTENTIALLY RESULTS IN DECREASED TRANSLATIONAL ACTIVITY IN THE ENTOMPATHOGENIC FUNGUS METARHIZIUM ACRIDUM

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Introduction

*M*etarhizium *acridum* is an important entomopathogenic fungus currently being used for biological control of insect pests. Environmental stressors such as ultraviolet radiation and heat require the fungus to be as stress-tolerant as possible. It was previously observed that visible light increases *M. acridum* tolerance to ultraviolet radiation and to heat.

Methods

We employ a combination of transcriptomics (mRNA-Seq) and high-throughput proteomics to understand how light regulates gene transcription and protein accumulation. Twenty-four-hour-old cultures grown in the dark were briefly exposed to visible light for 5 min and returned to dark conditions for different periods according to the technique employed: 0, 10, 25, 55, and 115 min for mRNA-Seq; and 10, 25, 55, 115, and 235 min for proteomics. Cultures kept in the dark (no light exposure) were used as controls (DD). Fold change was calculated relative to DD for each time point. Transcripts and proteins were considered regulated if their abundance changed at least two-fold relative to DD

Results and Discussion

Light exposure resulted in changes at the mRNA level for 1128 genes (11.3% of the genome). The number of proteins changing in abundance was only 57. Combining the two datasets, only 34 genes were regulated both at the transcript and the protein levels. Because only 34 transcripts/proteins were commonly regulated in both datasets, we were left with 23 proteins that changed in abundance in the absence of mRNA regulation and also 1094 regulated transcripts for which there was no protein change. Among down-regulated proteins, we observed subunits of eIF3, the eIF5A-modifying enzyme deoxyhypusine hydroxylase, and ribosomal proteins. This indicates that light reduces translational activity, which is one potential explanation for the reduced number of regulated proteins.

Conclusions

Taken together, our results indicate that while light regulates mRNA levels for many genes, it also reduces translational activity, thus making essential the study of protein levels in order to fully understand light response in fungi.

Acknowledgements

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> OC061. Oral Communication
Symposium PDT-11 Short Communications on antimicrobial PDT (Montserrat Agut)

FERTILE EGG SANITATION BY PHOTOACTIVATABLE PIGMENTS

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Background

Fertile poultry eggs used for hatching must withstand an assault of potentially pathogenic microorganisms from the moment the egg is laid until the chick has hatched. While the egg is fortified with an arsenal of antimicrobial defenses, some of these defenses remain unknown, uncharacterized, or underutilized. One such example is the photoactivatable protoporphyrin IX pigment found in brown eggshells. Preliminary research has shown that photoactivation of protoporphyrin IX exhibits antimicrobial effects. The objective of this research was to determine optimal conditions under which photoactivation of endogenous protoporphyrin IX and synthetic cuticle constituents result in reducing egg contamination by microorganisms. To address the potential use of photoactivation on eggs, we conducted three sets of experiments:

Methods

1. Eggs were inoculated with a lab strain of E. coli, treated with photoactivation protocols, and remaining bacteria were quantified by serial dilution and plating. Treatments included varying light intensity and wavelength, exposure duration, type and concentration of photoactivatable pigment, and presence of photoactivation potentiators.
2. Penetration of eggshells by bacteria and subsequent spoilage was promoted by scrambling egg yolks and immersing pre-warmed eggs in bacteria slurries followed by cooling. Chicken feces was used as a natural microbial inoculant, and Green Fluorescent Protein-expressing E. coli was used as a controlled microbial inoculant. Contamination was scored and quantified as a percentage of total eggs set.
3. 16s ribosomal rRNA was extracted from swabbed samples, sequenced, and classified into bacterial clades.

Bacterial reductions were expressed in log10 units and were evaluated for significance by two-way ANOVA for light and chemical treatments.

Results

Bacterial reductions of >4 logs were readily achievable under sufficient irradiance and exposure times. Endogenous protoporphyrin was more effective than exogenous, and exogenous TiO2 with potentiator was most effective (>6 logs). Gram-positive bacteria were more sensitive to photoactivation than gram-negative.

Conclusions

This work provides a foundation for continuous egg sanitation technology in poultry breeder farms and hatcheries.

> OC062. Oral Communication

Symposium PDT-11 Short Communications on antimicrobial PDT (Montserrat Agut)

PHOTODYNAMIC INACTIVATION OF ANTIBIOTIC-RESISTANT BACTERIA AND BIOFILMS WITH NANOMOLAR PHOTOSENSITIZER CONCENTRATIONS

Authors: Mariette Pereira[1], Carolina Vinagreiro[1], Amanda Zangirolami[2], Fabio Schaberle[1], Kate Blanco[2], Gabriela Jorge-da-Silva[3], Vanderlei Bagnato[2], Sandra Nunes[1], Alberto Pais[1], Luis Arnaut[1]
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Introduction

Gram-negative bacteria and bacteria in biofilms are very difficult to eradicate and are at the origin of the most antibiotic-resistant bacteria. Therapeutic alternatives less susceptible to mechanisms of resistance are urgently needed to respond to an alarming increase of nosocomial infections. Photodynamic inactivation (PDI) generates oxidative stress that triggers multiple cell death mechanisms more difficult to counteract by bacteria. Cationic photosensitizers have high phototoxicities towards Gram-negative bacteria but the challenge of inactivating multidrug-resistant strains and biofilms persists.

Methods

Novel cationic imidazolyl porphyrins were modelled with *ab initio* methods, synthesized, isolated and fully characterized, including their photochemical properties. PDI of planktonic *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, as well as of bacterial strains resistant to all beta-lactamic antibiotics and quinolones antibiotics collected at University of Coimbra Hospital Center (*S. aureus* and *Acinetobacter* collected from the skin of a burnt patient, *S. aureus* collected from an abdominal infection following surgery and *P. aeruginosa* strain from unknown origin also resistant to penicillins and gentamicin) were evaluated. Selected photosensitizers were screened for PDI of *S. aureus* biofilms.

Results and Discussion

We show how charge distribution in the photosensitizer impacts on the efficacy of inactivation of bacteria. We demonstrate the relevance of size for drug diffusion in biofilms. Designed *meso*-imidazolyl porphyrins of small size with positive charges surrounding the macrocycle enabled the inactivation of bacteria in biofilms by 6.9 log units at 5 nM photosensitizer concentration and 5 J/cm^2.

Conclusions

The unprecedented phototoxicity of small, cationic imidazolyl porphyrins offers new opportunities to treat biofilm infections.

Acknowledgements

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Conflicts of Interest

None

> OC063. Oral Communication

Symposium PDT-12 Short Communications in Chemical PDT (Kristian Berg)

CYCLODEXTRIN-BASED PHOTOACTIVE LIPOSOMAL NANOPARTICLES FOR TUMOR TARGETING

Authors: Ilya Yakavets[1,2,3], Henri-Pierre Lassalle[1,2], Vladimir Zorin[3,4], Lina Bezdetnaya[1,2]
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Introduction

Application of meta-tetra(hydroxyphenyl)chorin (mTHPC), one of the most potent photosensitizers, in the photodynamic therapy (PDT) of solid tumors encounters several complications resulting from its insolubility in aqueous media. The present study is aimed at the development of drug-in-cyclodextrin-in-liposome (DCL) nanoparticles by coupling two independent delivery systems: cyclodextrin/mTHPC inclusion complexes and liposomal vesicles to improve the transport of mTHPC to target tissue and to strengthen its intra-tissue accumulation in tumor. Liposomes offer an excellent opportunity to achieve selective drug targeting what is expected to prevent local irritation and reduce drug toxicity. Cyclodextrins (CDs) have been utilized as independent carriers for improvement of pharmaceutical properties such as solubility, stability, and bioavailability of various drug molecules, including mTHPC [1]. Therefore, we assumed that encapsulation of CD-complexed drug into liposomes may increase drug loading capacity, entrapment efficiency, may restrain the dissociation of drug-CD complexes, and prolong its systemic circulation.

Results and discussions

DCL nanoparticles have been prepared with various compositions to optimize the structure aiming to alter in a more favorable way the distribution of mTHPC in tumor tissue [2]. It was demonstrated that mTHPC-DCLs are stable and almost all mTHPC is bound to β-CDs in the inner aqueous liposome lumen. The influence of DCLs on mTHPC accumulation, distribution and photodynamic efficiency was studied in human adenocarcinoma HT29 cellular monolayer and spheroid models. Among all tested DCLs, double loaded DCL, which include mTHPC in lipid bilayer along with (CD-mTHPC) inclusion complexes in the inner aqueous lumen, displayed the highest potency for mTHPC delivery. Using 3D multicellular HT29 tumor spheroids we demonstrated that trimethyl-β-CD-based DCL provides homogenous accumulation of mTHPC across tumor spheroid volume thus supposing optimal mTHPC distribution.

Conclusions

DCL could circumvent the drawbacks of each separate system and could be used as a platform for mTHPC delivery. The data obtained confirm the interest in hybrid nanostructures for mTHPC-PDT.

Acknowledgements

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References

1. Yankovsky, I. *et al.*Inclusion complexation with β-cyclodextrin derivatives alters photodynamic activity and biodistribution of meta-tetra(hydroxyphenyl)chlorin. *Eur J Pharm Sci***91**, 172–182 (2016).
2. Yakavets, I. *et al.*Temoporfin-in-Cyclodextrin-in-Liposome—A New Approach for Anticancer Drug Delivery: The Optimization of Composition. *Nanomaterials***8**, 847 (2018).

> OC064. Oral Communication
Symposium PDT-12 Short Communications in Chemical PDT (Kristian Berg)

XYLAN-BASED NANOPARTICLES FOR TARGETED PHOTODYNAMIC THERAPY

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Photodynamic therapy (PDT) is an alternative and a minimally invasive cancer treatment requiring the simultaneous presence of three elements: photosensitive molecule, light source and molecular oxygen. This therapy involves intravenous administration of photosensitizers (PS), followed by local irradiation at an appropriate wavelength (generally red light). Irradiation of PS allows the production of reactive oxygen species (ROS) such as singlet oxygen or radicals, leading to cell death. The most used photosensitizers in PDT are porphyrins and their derivatives. However, these compounds often suffer from low solubility in physiological media and a lack of selectivity towards cancer cells which limits their clinical uses.

In order to overcome these problems, several therapeutic approaches are currently being studied. One of the most promising strategies is the use of nanoparticles as a vector of PS. These nano-objects can be designed to passively accumulate in tumor tissue via Enhanced Permeability and Retention (EPR) effect. For effective PDT, nanoparticles should ideally target specific organelles which are most sensitive to ROS such as mitochondria. In addition to production of energy, mitochondria play a crucial role in regulating cell death via apoptosis. Moreover, these nanoparticles must exhibit good biocompatibility and low toxicity, as is the case with polymeric nanoparticles currently in full development.

In this context, we have developed the synthesis of nanoparticles based on xylan for targeted delivery of porphyrins. Two types of nanoparticles have been studied: core-shell hybrid nanoparticles with a silica core and xylan-porphyrins shell functionalized with Triphenylphosphonium (TPP) as mitochondria targeting ligand and organic nanoparticles formed by self-assembly of xylan-porphyrins. Such xylan nanoparticles carrying a photosensitive drug are biocompatible and biodegradable. The polysaccharide creates a hydrophilic protective layer around the nanoparticles that help to increase half-life time of the nanoparticles in the blood circulation. In a first study the xylan-porphyrins were used as covering materials of silica nanoparticles (SiO2 NP). Indeed, the presence of glucuronic acids groups on xylan allows the formation of ionic bonds on the surface of the SiO2 NP made cationic by ammonium salts. In a second approach xylan-porphyrins were used alone to form nanoparticles fully organic by self-assembly in aqueous solution [1]. Different objects with variable degree of substitution in porphyrin have been obtained and characterized and their therapeutic potential for photodynamic therapy evaluated against colorectal cancer cell lines.

References

[1] S. Daus, T. Heinze, Macromolecular Bioscience, 2010, 10, 211–220.

> OC065. Oral Communication
Symposium PDT-12 Short Communications in Chemical PDT (Kristian Berg)

TOWARDS NANOSENSORS FOR THE SIMULTANEOUS MONITORING OF DIFFERENT INTRACELLULAR REACTIVE OXYGEN SPECIES: THE CHEMISTRY OF THE PROBES

Authors: Adrien Ratier[1], Francesca Giuntini[1], Gillian Hutcheon[1]
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Reactive oxygen species (ROS) play important roles for regulation of normal functions as proliferation, differentiation, migration and cell death. At low dose, they participate at the redox balance, but an excess of these molecules leads to damages on proteins, lipids or DNA [1,2]. ROS are involved in the onset and progression of several degenerative diseases (e.g., cancer, neurological disorder, etc). Cancer cells are highly susceptible to ROS-mediated damage and several chemotherapy agents achieve cytotoxicity by inducing oxidative stress.

Sensing the variations of different intracellular ROS is crucial for real time assessments of anticancer treatment efficiency. Yet, no sensor currently allows simultaneous and independent monitoring of different ROS live cells. Indeed, existing probes monitor either the total levels of ROS or the levels of single species (i.e., probes as diphenylanthracene, or peroxy yellow, or anthrafluorescein, etc.) [3–5].

The need for the optimization and the personalization of treatment regimens and for unravelling the mechanisms underpinning the still understood ROS-induced cell death, requires the introduction of a new set of tools able to provide a real-time report of intracellular ROS levels in response to a given intervention.

The aim of this project is to synthetize new fluorescent probes with functional moieties to graft them on nanoparticles. We will discuss the synthetic approaches to new conjugatable molecular sensors for different ROS, the way to graft same on polymers based on poly(lactic-co-glycolic acid) and, finally, the synthesis of nanoparticles by different ways (nanoprecipitation and microfluidic system)[6,7]. Different analysis will be made on those new probes at different steps (molecules, polymers or nanoparticles) to show their selectivity, their efficiency and their injection in cells.

References

(1) Schumacker, P. T. Reactive Oxygen Species in Cancer Cells: Live by the Sword, Die by the Sword. *Cancer Cell* **2006**, *10* (3), 175–176.
(2) Sena, L. A.; Chandel, N. S. Physiological Roles of Mitochondrial Reactive Oxygen Species. *Mol. Cell* **2012**, *48* (2), 158–167.
(3) Gomes, A.; Fernandes, E.; Lima, J. L. F. C. Fluorescence Probes Used for Detection of Reactive Oxygen Species. *J. Biochem. Biophys. Methods* **2005**, *65* (2–3), 45–80.
(4) Dickinson, B. C.; Srikun, D.; Chang, C. J. Mitochondrial-Targeted Fluorescent Probes for Reactive Oxygen Species. *Curr. Opin. Chem. Biol.* **2010**, *14* (1), 50–56.
(5) Wang, H. S. Development of Fluorescent and Luminescent Probes for Reactive Oxygen Species. *TrAC - Trends Anal. Chem.* **2016**, *85*, 181–202.
(6) Crucho, C. I. C.; Barros, M. T. Formulation of Functionalized PLGA Polymeric Nanoparticles for Targeted Drug Delivery. *Polymer (Guildf).* **2015**, *68*, 41–46.
(7) Nie, Z.; Li, W.; Seo, M.; Xu, S.; Kumacheva, E. Janus and Ternary Particles Generated by Microfluidic Synthesis: Design, Synthesis, and Self-Assembly. *J. Am. Chem. Soc.* **2006**, *128* (29), 9408–9412.

> OC066. Oral Communication
Symposium PDT-12 Short Communications in Chemical PDT (Kristian Berg)

DNA-ACTIVATABLE PHOTOSENSITIZERS BASED ON BIOORTHOGONAL REACTIONS

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Photodynamic therapy (PDT), is a medical treatment based on the generation of cytotoxic reactive oxygen species (ROS) such as $^1O_2$ upon irradiation of light-sensitive chemicals known as photosensitizers (PS). Although light stimulation offers the advantage of precise localization of the irradiation on the disease side, major problems are still the dark toxicity of classical PSs, photosensitivity and off-target effects due to PSs accumulation in healthy tissues. To overcome these limitations, two different strategies related to novel PSs have been currently used: either activatable[1] or specifically delivered[2] ones. Here, we introduce a new strategy to simultaneously achieve both; i.e. conditional phototoxicity and specific subcellular PS localization. Thus, our dormant PS shows a significant cytotoxic enhancement upon DNA-activation via a bioorthogonal reaction. Importantly, to our knowledge, this is the first time that bioorthogonal reactions are used in the context of photodynamic therapy. We believe that our novel strategy becomes an important step towards smart photodynamic methodologies.

References

[1] *Chem. Soc. Rev.*, **2010**, *110*, 2839-2857. [2] *Anticancer. Res.*, **2013**, *33*, 2823-2831.

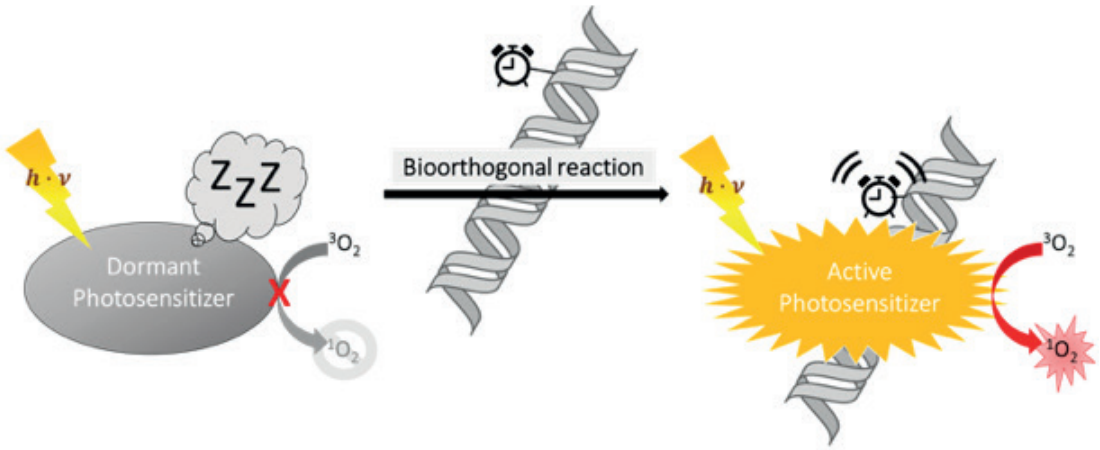

Figure 1. Outline of the principle of our DNA-targeted strategy.

> OC067. Oral Communication
Symposium PDT-12 Short Communications in Chemical PDT (Kristian Berg)

NOVEL RU(II) POLYPYRIDYL COMPLEXES AS PHOTODYNAMIC THERAPY PHOTOSENSITIZERS

Authors: Marta Jakubaszek[1,2], Bruno Goud[2], Gilles Gasser[1]
Presenting Author: Marta Jakubaszek
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Photodynamic therapy (PDT) is a medical technique, which can be used as an alternative or complimentary treatment to radiotherapy, chemotherapy and surgery. PDT relies on the combination of an ideally non-toxic photosensitizer (PS), oxygen and light. The PS is administrated into the patient, and treated tissue is irradiated at a specific and defined wavelength. The PS is then activated to produce reactive oxygen species (ROS), which leads to impairment of metabolic pathways and ultimately, cell death. However, the currently used PSs have a number of drawbacks, namely low solubility in water, photobleaching, low cancer selectivity and slow clearance from the patients bodies that leads to photosensitivity. The search for new photosensitizers that are specific for tumour tissue, have no cytotoxicity in the dark and which can be activated via higher wavelengths, for deeper penetration through tissue, led us to investigate Ru(II) polypyridyl complexes.[1, 2] Among the different complexes studied in our lab, one was found to be especially potent. We were able to target it to the cancer cells thanks to the attachment to a biomolecule. We will present our latest results during this conference.

References

1. F. Heinemann, J. Karges and G. Gasser, *Acc. Chem. Res.*, 2017, **50**, 2727-2736.
2. M. Jakubaszek, B. Goud, S. Ferrari and G. Gasser, *Chem. Commun.*, 2018, **54**, 13040-13059.

> OC068. Oral Communication
Symposium PDT-12 Short Communications in Chemical PDT (Kristian Berg)

DEVELOPMENT OF ORGANELLE-TARGETING PHOTOSENSITIZERS

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With more than 14 million reported new cases and about 9 million deaths per year, cancer represents the hardest therapeutic challenge worldwide. Reactive species are a main cause for carcinogenesis, but are also widely used for cancer eradication. Hydrogen peroxide, hydroxyl radical, singlet oxygen ($^1O_2$), and other reactive species are commonly produced by anticancer therapeutics and are responsible not only for destruction of malignancies, but also for unwanted side effects. The best way to increase the efficacy and to limit the side effects of a drug, is to deliver it to a specific target. Cancerous cells commonly display alerted metabolism and preferential uptake of certain compounds, among them porphyrins. Preferential accumulation of porphyrins in cancerous tissues has been used for a long time for tumor imaging. Porphyrin uptake by cancer cells is of special interest because porphyrins can act as photosensitizers (PS), absorbing energy of visible light and generating reactive species capable of killing cells.

Singlet oxygen is considered the principle factor causing damage to critical cellular components, which ultimately leads to cell death. Since $^1O_2$ has short life in biological environment, damage is limited to the close proximity of the porphyrin molecule. Different cellular organelles and structures show dramatic differences in sensitivity to $^1O_2$. Location and extent of damage trigger signaling pathways, which define cellular responses and mechanisms of cell death. The mode of cell death in turn determines the overall organismal response. Among the organelles that are particularly attractive as targets for such therapy are the mitochondria. They play a key role in energy production and in initiation and execution of cell death mechanisms. Design of mitochondria-targeting PSs by rational molecular synthesis is still underdeveloped. In general, two techniques are used to give a PS molecule mitochondria-targeting ability: (1) Attachment of a mitochondria-targeting peptide sequence; and (2) Combination of lipophilic residues with cationic groups, thus exploiting the high membrane potential across the inner mitochondrial membrane. Investigations with porphyrin-based PSs revealed that in addition to overall charge and lipophilicity, other molecular parameters are critical for targeted delivery of a PS molecule to desired organelle. Among them are position and accessibility of charges, three-dimensional shape of the molecule, its flexibility, bulkiness, and size. Proper selection and combination of these parameters is essential for targeted organelle delivery.

> OC069. Oral Communication
Symposium PDT-12 Short Communications in Chemical PDT (Kristian Berg)

DEVELOPMENT OF TARGETED NEAR INFRARED AG2S QUANTUM DOTS FOR OPTICAL IMAGING AND PHOTODYNAMIC THERAPY

Authors: Mahshid Hashemkhani[koc u], Havva Yagci Acar[koc u], Layla Mohammad Hadi[UCL u], Alexander J. MacRobert[UCL u], Marilena Loizidou[UCL u]
Presenting Author: Mahshid Hashemkhani
1) koc university

Introduction

Cancer is one of the leading cause of death worldwide. Advanced methods for its early detection and more efficient therapies are needed [1], [2]. Discovery of advanced materials and new imaging techniques serving for simultaneous diagnosis and therapy provided new opportunities [3]. Here, we will demonstrate the use of molecularly targeted Ag2S quantum dots for optical imaging, carrying a photosensitizer pro-drug 5-aminolevulinic acid (ALA) for site specific photodynamic therapy (PDT). We will also discuss the time and cell line dependence of ALA to PpIX conversion as well as the mode of ALA conjugation to QDs for most efficient PpIX generation in vitro.

ALA was conjugated to cyto/hemocompatable Ag_2S-2MPA NIRQDs via different methods in order to investigate the impact of conjugation method on PpIX generation, dark cytotoxicity and PDT potential. PpIX generation was studied as a function of time and cell lines. PDT and combination therapy potential of these QDs conjugated with cetuximab will be also discussed.

Methods

Ag_2S-2MPA QDs were prepared in water from Na2S and AgNO3. Ala was either electrostatically loaded to anionic Ag_2S-2MPA QDs or conjugated covalently via an amide or hydrazone bond. ALA release and PpIX generation was studied using a microplate reader after 24 and 48h incubation of free ALA and ALA loaded QDs with the cancer cell lines (excitation: 420nm, emission: 635nm). Cytotoxicity was evaluated with MTT or Alamar Blue assays before/after ALA conjugation and 5-10 min laser irradiation of treated cells at 630 nm. In vitro optical imaging was performed using a fluorescent microscope.

Results and discussions

Aqueous colloidal Ag_2S-2MPA QDs with excellent stability was achieved with emission maxima between 750-850 nm. ALA to PpIX conversion studied in HeLa, MCF7, Caco2, HT116 and HT29, indicated that HT29 and Caco2 are the most efficient ones and HT116 is the poorest. QDs were well internalized by HT29 and HeLa cells and provided strong optical signal in the NIR. QDs showed no cytotoxicity up to 200 µg/ml Ag concentration in either cell lines but induced some dark toxicity when conjugated with ALA. The electrostatic conjugation of ALA to the QDs results in higher PpIX generation compared to free ALA and the other conjugates. We will also discuss the PDT potential of these QDs in vitro.

References

[1] J. Zhang,"Folic acid-conjugated green luminescent carbon dots as a nanoprobe for identifying folate receptor-positive cancer cells," *Talanta*
[2] H. Wu, X. Zhu, "A novel near-infrared triggered dual-targeted nanoplatform for mitochondrial combined photothermal-chemotherapy of cancer in vitro," *Nanotechnology*
[3] D. Asik,"One step emission tunable synthesis of PEG coated Ag_2S NIR quantum dots and the development of receptor targeted drug delivery vehicles thereof," *J. Mater. Chem. B*, vol. 4, no. 11, pp. 1941–1950, 2016.

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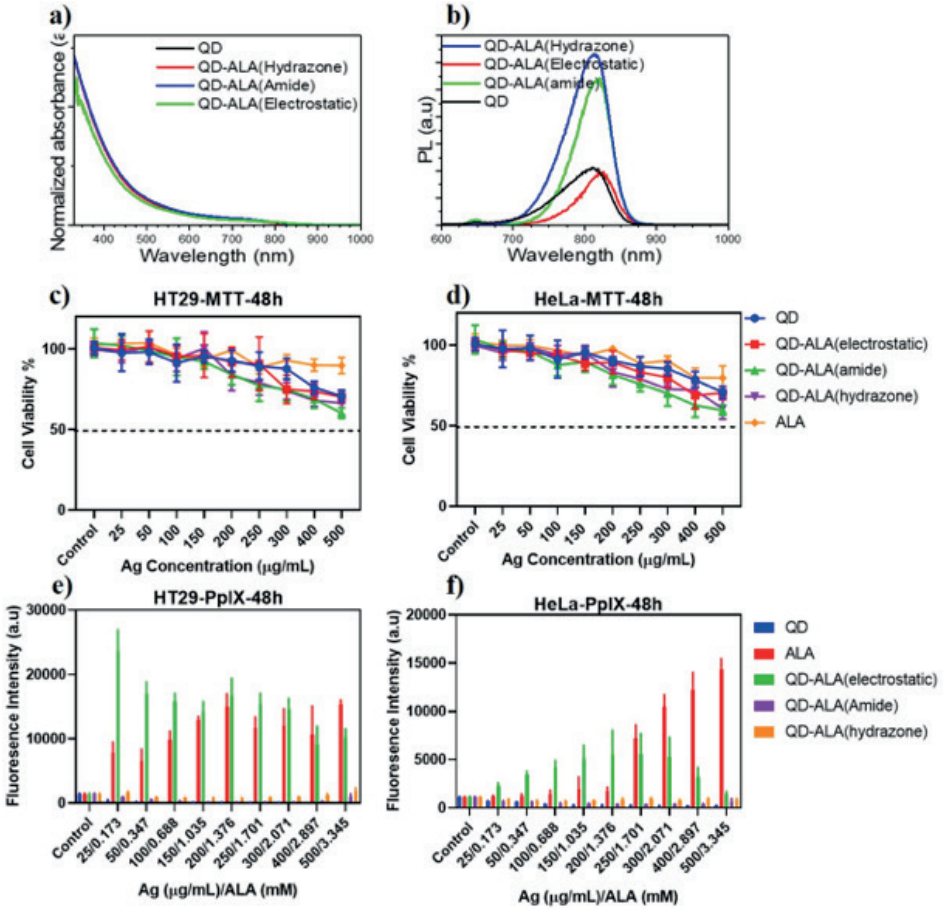
a)
Normalized absorbance (ε
QD
QD-ALA(Hydrazone)
QD-ALA(Amide)
QD-ALA(Electrostatic)
Wavelength (nm)
b)
PL (a.u)
QD-ALA(Hydrazone)
QD-ALA(Electrostatic)
QD-ALA(amide)
QD
Wavelength (nm)
c)
HT29-MTT-48h
Cell Viability %
Ag Concentration (μg/mL)
d)
HeLa-MTT-48h
Cell Viability %
Ag Concentration (μg/mL)
QD
QD-ALA(electrostatic)
QD-ALA(amide)
QD-ALA(hydrazone)
ALA
e)
HT29-PpIX-48h
Fluoresence Intensity (a.u)
Ag (μg/mL)/ALA (mM)
f)
HeLa-PpIX-48h
Fluoresence Intensity (a.u)
Ag (μg/mL)/ALA (mM)
QD
ALA
QD-ALA(electrostatic)
QD-ALA(Amide)
QD-ALA(hydrazone)

> OC070. Oral Communication
Symposium PDT-12 Short Communications in Chemical PDT (Kristian Berg)

CARBOXAMIDE BACTERIOCHLORINS AS NOVEL PREFORMED PHOTOSENSITIZERS FOR TOPICAL PDT OF SKIN DISORDERS

Authors: Helder Soares[1,2], Sergio Simoes[2,3], Luis Arnaut[1]
Presenting Author: Helder Soares
1) CQC and Department of Chemistry, University of Coimbra, 3004-535 Coimbra, Portugal 2) Center for Neuroscience and Cell Biology University of Coimbra, 3004-534 Coimbra, Portugal 3) Luzitin SA, Edificio Bluepharma, S. Martinho do Bispo, 3045-016 Coimbra, Portugal

Introduction

The current PDT approach to skin disorders is the topical administration of aminolevulinic acid, a precursor in the biosynthesis of protoporphyrin IX, followed by the illumination of PpIX 3-4 h post-administration of the prodrug. This is a lengthy and inefficient procedure aggravated by the weak absorption of PpIX (molar extinction coefficient 5000 $M^{-1}cm^{-1}$at 630 nm), which limits the treatment to superficial skin lesions.

Topical delivery attempts with pre-formed photosensitizers such as hypericin, silicon phthalocyaninea and temoporfin did not reach meaningful results since the flux of a drug across the skin is strongly limited by its molecular weight.

Progress in PDT of skin lesion depends on the availability of more potent photosensitizers, with strong light absorption in the near infrared (where the skin is more transparent), that rapidly and efficiently permeate the skin.

Results and discussions

In this work we report the first low molecular weight and photostable carboxamide bacteriochlorin, with strong absorption in the NIR, amphiphilicity appropriate for skin permeation, a fast uptake and tropism to the Endoplasmic reticulum and to the Golgi apparatus and remarkable phototoxicity (picomolar concentrations @10J/cm^2) against several cancer cell lines and also against bacterial strains (eg. *P. Acnes*).

Minipig skin permeation of a topically applied water-based formulation showed significant amounts of drug at more than 50mm after 2hours contact. Time can be dramatically improved by employing active methods of permeation like piezoporation.

When applied to mice bearing CT26 and B16F10 *sc t*umors it significantly impacted the kinetics of tumor regrowth. Also, the pharmacokinetics shows a fast clearance from the body (t1/2 ~3h) diminishing the risk of off-target reactions.

Conclusion

The efficient synthesis of a photostable low molecular weight carboxamide bacteriochlorin offers the possibility to treat deep lesions with topical administration. Our results show that this carboxamide bacteriochlorin is an excellent candidate for PDT of skin lesions.

Acknowledgments

This work was financially supported by the Portuguese Science Foundation (007630UID/QUI/00313/2013, PTDC/QEQ-MED/3521/2014, Roteiro/0152/2013/022124) and by QREN/30218. Helder T. Soares thanks FCT for grant SFRH/BD/119058/2016. We acknowledge EC support through Laserlab-Europe, H2020 EC-GA 654148. We very especially thank Luzitin SA for continued support.

> OC071. Oral Communication

Symposium PDT-13 Short Communications on Cancer PDT (M. Lluïsa Sagristá)

DYNAMIC PHOTOPHYSICS OF PORPHYRIN-LIPID LIPOSOMES FOR THERANOSTICS

Authors: Danielle M. Charron[1,2], Hilde H. Buzzá[3], Maneesha A. Rajora[1,2], Miffy H.Y. Cheng[1], Juan Chen[1], Gang Zheng[1,2]
Presenting Author: Danielle M Charron
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Introduction

Photosensitizers are inherently responsive to their environment; harnessing this responsivity is the key to generating dynamic optical agents. Supramolecular assemblies of photosensitizers are responsive to stimuli that alter their structure and change the extent and nature of dye aggregation. Here, we explore the aggregation-dependent photophysics of porphyrin-lipid liposomes and provide recommendations for their theranostic uses.

Methods

To take advantage of the collective behaviour of lipid membranes, bacteriopheophorbide *a* was conjugated to phosphatidylcholine in place of a hydrocarbon tail. Porphyrin-lipid optical properties and therapeutic capabilities were measured with respect to host lipid saturation and temperature. Membrane order and hydration were evaluated using fluorescent probes. Porphyrin alignment was assessed by circular dichroism spectroscopy and NMR.

Results and Discussion

Within saturated host lipid liposomes, porphyrin-lipids J-aggregate; their dipole moments align head-to-tail, causing their Q_y absorption to red-shift from 755 nm to 824 nm and intensify. At room temperature, J-aggregated porphyrin-lipid liposomes are quenched >95% with respect to fluorescence and singlet oxygen generation, making them efficient photothermal and photoacoustic agents. The maximum temperature reached by laser irradiation is ~50 °C, lower than both the absorption transition at 52 °C and the host lipid (DSPC) transition at 55 °C. At 50 °C, J-aggregation improves due to increased membrane fluidity, enhancing fluorescence 2-fold. At 52 °C, the absorption peak reverts to that of the monomer as porphyrin alignment is lost. Within unsaturated host lipid liposomes, porphyrin-lipids retain their monomer absorption and are fluorescently quenched. However, singlet oxygen generation is only ~70% quenched and the porphyrins rapidly photobleach. This dramatic difference from the J-aggregated variant is related to increased lipid hydration caused by porphyrin-lipid inclusion.

Conclusions

Porphyrin-lipid liposomes exhibit membrane-dependent and temperature-responsive properties that can be applied for new theranostic applications. The discrepancy between photothermal, absorption, and host lipid transition temperatures of J-aggregated variants highlights the challenges of rationally designing these agents for phototherapy and the importance of measuring the temperature dependence of all deactivation pathways. Porphyrin-lipid also impacts the liposome phase, complicating extrapolation of known photosensitization capabilities of porphyrins in liposomes to porphyrin-lipid. From a nanomedicine development viewpoint, we found fluorescence to be an unreliable surrogate for singlet oxygen quenching. Future studies will extend the membrane and photosensitizer compositions studied and investigate the impact of protein adsorption.

Acknowledgements

This study was funded by NSERC, CIHR, CFI, TFRI, and PMCF.

Conflicts of Interest

None.

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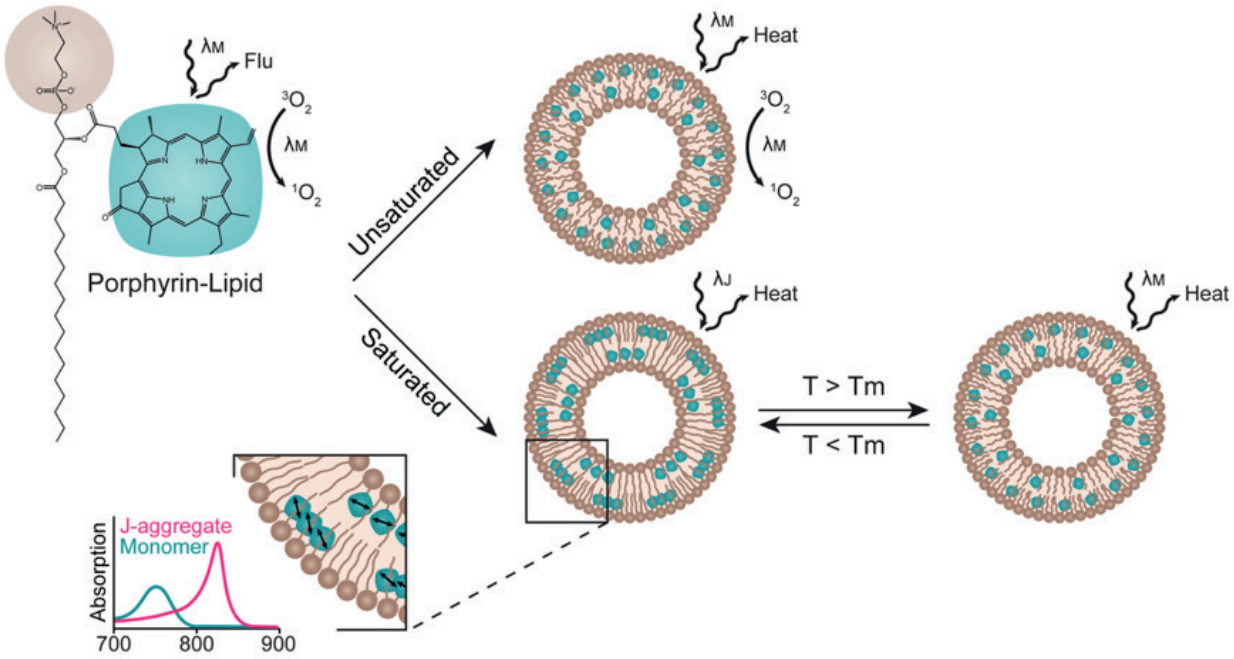
λM
Flu
$^3O_2$
λM
$^1O_2$
Porphyrin-Lipid
Unsaturated
Saturated
λM
Heat
$^3O_2$
λM
$^1O_2$
λJ
Heat
T > Tm
T < Tm
λM
Heat
Absorption
J-aggregate
Monomer
700
800
900

> OC072. Oral Communication
Symposium PDT-13 Short Communications on Cancer PDT (M. Lluïsa Sagristá)

IMAGING GUIDED NANOFORMULATIONS WITH CONTROLLED EXCITATION DYNAMICS TO ENHANCE PHOTODYNAMIC THERAPY

Authors: Tymish Ohulchanskyy[1], Oksana Chepurna[1], Hao Xu[1], Roman Ziniuk[1], Ludmyla Vretik[2], Yuri Slominskii[3], Junle Qu[1]
Presenting Author: Tymish Y. Ohulchanskyy
1) Shenzhen University, Shenzhen, Guangdong, China 2) Taras Shevchenko National University of Kyiv, Kyiv, Ukraine 3) Institute of Organic Chemistry NASU, Kyiv, Ukraine

Over the last few years, we have been working on inorganic and organic nanomaterials for optical bioimaging and imaging guided photodynamic therapy. A nanochemistry approach allows us to combine therapeutic and imaging agents and fabricate nanoparticles for targeted, imaging guided delivery of PDT drugs (photosensitizers, PS) to cancer sites. Light induced electronic processes play a key role in the functionality of these photoactive nanoplatforms; their imaging and therapeutic functionalities can be tuned and optimized through control of PS excitation dynamics and specific electronic processes within nanoparticles (e.g., electronic excitation energy transfer). The talk will provide examples of the nanoformulations, where electronic processes have been orchestrated with the intent to enhance imaging and PDT functionalities. The developed nanostructures include liposomal and polymeric nanoparticles, near-infrared fluorescent organic dyes, rare-ion doped nanophosphors, as well as their hybrids.

While possessing the optical imaging contrast and PDT functionality, the light active nanoplatforms can be also garnished with other medical imaging modalities, enabling the integration of cellular, tissue and whole body imaging and allowing us to employ a single nanoagent for multiple imaging techniques. The talk will demonstrate examples of applications of nanoparticles as multimodal imaging guided PDT agents and conclude with a discussion on the challenges and opportunities in the field of nanoformulations for imaging guided photodynamic therapy.

> OC073. Oral Communication

Symposium PDT-13 Short Communications on Cancer PDT (M. Lluïsa Sagristá)

NEW PLATFORMS FOR IMPROVING THE TREATMENT OF GLIOBLASTOMA BY PHOTODYNAMIC THERAPY

Authors: Ludivine Larue[1,2], Ludovic Colombeau[1], Philippe Arnoux[1], Francis Baros[1], Gerard Audran[3], Sylvain R.A. Marque[3], Cedric Boura[4], Samir Acherar[2], Celine Frochot[1]
Presenting Author: Ludivine Larue
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Introduction

Glioblastoma is a grade IV tumor, recognized as the most malignant and aggressive brain tumor. The standard therapy is most often composed of three treatment modalities: surgery, chemotherapy and radiotherapy but it remains insufficient. Photodynamic therapy (PDT) is a promising technique for the treatment of some diseases including various malignant tumors[1]. A clinical trial for the treatment of glioblastoma by PDT is in progress in France (ClinicalTrials.gov Identifier: NCT03048240). However, PDT suffers from two major drawbacks: the lack of photosensitizer (PS) selectivity and the need of oxygen (O_2) to be efficient. The targeting of tumor neovessels is a promising approach to create damages by PDT. Our team has already proved the good affinity of KDKPPR peptide for neuropilin-1 receptor over-expressed onto endothelial cells.[2] The team of Audran and al.[3] has developed the alkoxyamines as theranostic agents. These molecules are able to release, after chemical activation, alkyl radicals that are toxic for cancer cells. Scaiano et al.[4] described the possibility of the release of these radicals by photo-activation. Our goal is to elaborate a multifunctional platform composed of a targeting peptide for tumor selectivity, a PS for PDT treatment and a photolabile alkoxyamine for the release of radicals even without O_2.

Results and Discussion

To achieve this goal, we have considered combining a photolabile alkoxyamine (O_2-independent compound), a PS (O_2-dependent compound, pyropheophorbide a) and a targeting peptide (KDKPPR) to obtain a targeted PDT effect in the presence or absence of O_2. As far as we know, the use of alkoxyamine to increase the PDT effect has never been studied as well as the combination of an alkoxyamine with a PS. A first series of compounds has been synthesized with success and the photophysical properties and the photolability of alkoxyamine have been studied. The proof of concept, that the combination of the alkoxyamine and the PS did not inhibit their respective ability to produce singlet oxygen and alkyl radicals, has been established.

Perspectives

The development of a new series of compounds is under progress. We plan to use another penetrating peptide and another alkoxyamine, which could be irradiated at longer wavelength. Photophysical properties analysis, photolability and biological studies will be performed.

References

1. Hamblin, M. *Advances in Photodynamic Therapy: Basic, Translational, and Clinical*. (Artech House, 2008).
2. Thomas, E. *et al.* Ultrasmall AGuIX theranostic nanoparticles for vascular-targeted interstitial photodynamic therapy of glioblastoma. *Int. J. Nanomedicine* **12**, 7075–7088 (2017).
3. Audran, G. *et al.* Alkoxyamines: a new family of pro-drugs against cancer. Concept for theranostics. *Org Biomol Chem* **12**, 719–723 (2014).
4. Scaiano, J. C. *et al.* Fluorescence sensor applications as detectors for DNA damage, free radical formation, and in microlithography. *Pure Appl. Chem.* **77**, 1009–1018 (2005).

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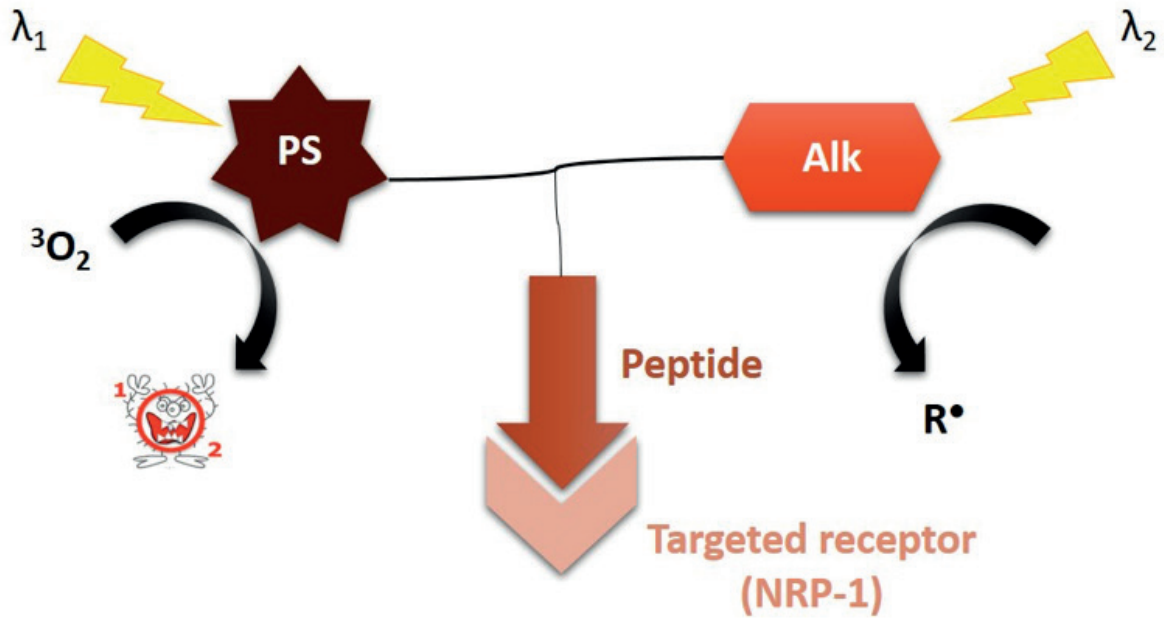
λ1
λ2
PS
Alk
3O2
Peptide
R•
Targeted receptor
(NRP-1)

> OC074. Oral Communication

Symposium PDT-13 Short Communications on Cancer PDT (M. Lluïsa Sagristá)

IN VITRO CHARACTERIZATION OF PORPHYRIN-BASED PHOTOSENSITIZERS

Authors: Irene Jiménez Munguía[1], Ivan Meshkov[2], Kirill Birin[2], Yulia Gorbunova[2,3], Valerij Sokolov[2]
Presenting Author: Irene Jiménez Munguía
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Introduction

Photodynamic therapy, commonly applied to treat skin cancer, is based on activation of photosensitizers (PS) by light delivery to generate reactive oxygen species, basically singlet oxygen (SO), damaging cell membrane

Methods

We studied *in vitro* the processes involved in photodynamic therapy on a model bilayer lipid membranes (BLM) by measuring the boundary potential applying the Intramembrane Field Compensation (IFC) Method (Sokolov and Kuz'min, Biofizika, 25:170, 1980). IFC method allowed to monitor binding of PS on BLM and damage of target molecules (TM) of SO under illumination. di-4-ANEPPS was used as TM since it adsorbs on BLM creating a dipole potential. The rate of TM oxidation was calculated from the kinetics of the potential drop under illumination and its restoration in dark

Results and Discussion

We studied the adsorption and photodynamic efficiency of newly synthesized positively charged porphyrins, namely b-imidazolyl substituted porphyrin and it's Zn(II) and In(III) complexes; and two phosphorus (V) complexes of *meso*-(*p*-pyridyl)-triphenylporphyrin bearing hydroxyl and ethoxyl axial ligands. We observed the adsorption of these PS on BLM by measuring the boundary potential change, which was proportional to the logarithm of concentrations of each compound. To evaluate their photodynamic efficiency, we determined the rate of oxidation (*R*) of TM adsorbed either in the same or opposite surface of the BLM where molecules of PS were adsorbed. Similar *R* values were obtained suggesting equal distribution of SO between two sides of BLM.

Conclusions

The adsorption of PS compounds on BLM was found to be a main factor influencing on the photodynamic efficiency of the porphyrin-based PS used in this study.

Acknowledgements

This work was supported by the Russian Foundation of Basic Research (N 19-04-00694a) and the Ministry of Education and Science of the Russian Federation *in the framework of* Increase Competitiveness Program of NUST «MISiS» (№ К4-2017-053).

> OC075. Oral Communication

Symposium PDT-13 Short Communications on Cancer PDT (M. Lluïsa Sagristá)

IN VIVO TWO-PHOTON IMAGING OF GENETICALLY ENCODED FLUORESCENT BIOSENSORS UNVEILS CONNEXIN-DEPENDENT SIGNALING PATHWAYS STIMULATED BY PHOTODYNAMIC THERAPY

Authors: Chiara Nardin[1,2], Chiara Peres[2], Flavia Mazzarda[2,3], Francesco di Virgilio[4], Marcello Raspa[2], Anna Maria Salvatore[2], Fabio Mammano[1,2]
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The signaling pathways engaged by photodynamic therapy (PDT) in treated cancer cells and the molecular mechanisms underlying concurrent bystander effects remain incompletely understood.

By two-photon intravital imaging of genetically encoded fluorescent biosensors, we detected in real time PDT-induced intraorganellar Ca^{2+} signals and the activation of apoptotic processes in the solid tumor *in vivo*.

Biosensor-expressing tumors were grown inside the dorsal skinfold chamber surgically implanted on the mouse back. *In vivo* PDT with the photosensitizer Aluminum Phthalocyanine Chloride (AlClPc) was performed, while simultaneously performing intravital multiphoton microscopy. Focal AlClPc photoactivation[1] was promoted by a 671 nm laser beam, focused in a 10 μm diameter spot for photostimulation of a single tumor cell at $2{\cdot}10^6$ mW/cm^2 irradiation fluency. Using targeted and selective biosensors, we monitored subcellular Ca^{2+} dynamics in the cytosol, endoplasmic reticulum and mitochondria of tumor cells. We also visualized the activation of caspases in the irradiated and bystander cells within seconds of AlClPc photoactivation. Since a significant role in intercellular communication and photodamage propagation is played by connexins-made channels (i.e. gap junction channels and unopposed hemichannels), we investigated the effect of connexin function on PDT-dependent bystander effects in the tumor.

In summary, the established experimental protocol allowed us to study bystander effects elicited by focal PDT in the solid tumor and the role of connexin signaling in cytotoxic stimuli transmission.

Supported by the Italian Ministry of Health, Project Code RF-2011-02348435 (P.I. FDV).

Reference

[1] Calì, B., Ceolin, S., Ceriani, F., Bortolozzi, M., Agnellini, A. H., Zorzi, V., ... & Mammano, F. (2015). Critical role of gap junction communication, calcium and nitric oxide signaling in bystander responses to focal photodynamic injury. *Oncotarget*, *6*(12), 10161.

> OC076. Oral Communication

Symposium PDT-13 Short Communications on Cancer PDT (M. Lluïsa Sagristá)

QUANTIFICATION OF DOXORUBICIN IN ENDOSOMES OF RAT BLADDER CANCER CELLS (AY27) BY PHOTOCHEMICAL INTERNALIZATION

Authors: Odrun A Gederaas[1], Orjahan Saidshahagha[1], Anders Høgset[3], Lars Hagen[1,4]
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Introduction

Doxorubicin (Dox) has been used for long time as an anticancer agent, but clinical effects of Dox are limited by its dose-related acute cardiotoxicity1 and acute multidrug resistance2. Because of that, it is of great interest to lower the concentration of the active substance. The novel photochemical internalization (PCI) technology3 are relevant for such a process by increasing cytosolic concentrations, even with lower incubation doses.

Methods

Doxorubicin (Dox) in endosomes of rat bladder cancer cells (AY27) are studied by photochemical internalization (PCI), a novel technology for cytosolic delivery of macromolecules based on photodynamic therapy (PDT). The AY27 cells were grown in standard RPMI growth medium and further incubated with the photosensitizer, meso-tetraphenyl chlorine disulphonate, TPCS2a (Amphinex®) followed by Dox stimulation and blue light illumination (LumiSource, 13mW/cm^2).

Results and discussion

Results shows a higher concentration of Dox in endosomes from AY27 cells internalized by PCI technology compared to cells with only Dox alone, all related to controls without any treatment.

Conclusions

The study presents a novel and exact protocol for determination of Dox in endosomes, a method which may be relevant for all kind of macromolecules, entrapped by photochemical internalization.

Acknowledgements

Grants from the Cancer Research Foundation of St. Olav's University Hospital, Trondheim, Norway.

Conflicts of Interest:

No 2

References

1. Tacar O, Sriamornsak P and Dass CR. J Pharm Pharmacol. 2013 Feb;65(2):157-70.
2. Carvalho C, Santos RX, Cardoso S, Correia S, Oliveira PJ, Santos MS, Moreira PI. Curr Med Chem. 2009;16(25):3267-85.
3. Berg K, Selbo PK, Prasmickaite L, Tjelle TE, Sandvig K, Moan J, Gaudernack G, Fodstad O, Kjølsrud S, Anholt H, Rodal GH, Rodal SK, Høgset A. Cancer Res. 1999 Mar 15;59(6):1180-3.

> OC077. Oral Communication

Symposium PDT-13 Short Communications on Cancer PDT (M. Lluïsa Sagristá)

COMBINATION THERAPY WITH METHYLENE BLUE BASED PHOTODYNAMIC AND RUTOSIDE FOR MELANOMA CANCER CELLS

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Melanoma is malignant form of skin cancer and is associated with a high mortality rate. Therefore, early diagnosis and surgical treatment is very important. Photodynamic therapy (PDT) involves the activation of a photosensitizer by light at specific wavelength that interacts with oxygen and produces singlet oxygen molecules or radical oxygen species (ROS) which leads to tumor cell death. In addition, one of the important strategies for the prevention and treatment of various cancers is the use of plant compounds. Phenolic compounds are important category of natural antioxidants which have important biological activities, such as anticancer effects. The aim of this study was to investigate the effects of combination therapy with methylene blue (MB) assisted photodynamic treatment (PDT (with a red light source (660 nm;power density: 30mW/cm^2)) and Rutoside (Rutin) as polyphenol (flavonoid) agent on human melanoma cancer cells. For this purpose, the human melanoma cancer cell line treated with MB-PDT and Rutoside. After treatment, MTT assay, clonogenic cell survival, cell death mechanisms such as autophagy and apoptosis were determined. Cell cycle distribution after photodynamic therapy (PDT) and also intracellular reactive oxygen species (ROS) generation were measured. The result showed that MB-PDT and Rutoside has better cytotoxic and antiprolifrative effect on A375 human melanoma cancer cells in compare to each drug alone while the effect on human normal cell is not significant. MB-PDT and Rutoside combination induced apoptosis, and cell cycle arrest in human melanoma cancer cell line. Intracellular ROS increased in A375 cancer cell line after treatment with MB-PDT and Rutoside. The results suggest that MB-PDT and Rutoside could be considered as a novel approach in the combination treatment of melanoma cancer.

> OC078. Oral Communication
Symposium PDT-13 Short Communications on Cancer PDT (M. Lluïsa Sagristá)

PRECLINICAL TREATMENT PLAN DEVELOPMENT FOR INTRAOPERATIVE PHOTODYNAMIC THERAPY USING A NOVEL OPTICAL SURFACE APPLICATOR

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Introduction
We are developing a treatment planning method for intraoperative photodynamic therapy (IO-PDT). Effective IO-PDT depends on accurate light dosimetry, including irradiance and fluence. Recently, we presented a novel surface applicator (OSA) to deliver light to large surface areas like the pulmonary pleura. Here, we describe the development of a treatment planning method for simulating the light delivery by the OSA.

Methods
Modeling the OSA was developed using the software Paraview [1], Python [2], and the Visualization Toolkit (VTK) [3]. Light simulations were performed in the Monte Carlo simulation package, FullMonte [4]. Simulations were validated by (i) analyzing digital images of the activated OSA and (ii) mapping viability of cultured A549 human lung carcinoma cells after incubation with a ruthenium-based photosensitizer [5] followed by exposure to 630-nm light from the OSA.

Results and Discussion
The script for modeling the OSA is capable of any size, with our prototype size, 10 cm x 10 cm, generated with an estimated maximum run time of 10 minutes. Fluence maps acquired from Monte Carlo modeling showed similarity between light distribution from digital images and pattern of cell viability.

Conclusion
The OSA model can provide accurate light dosimetry. We propose using this modeling technique in combination with our OSA for treatment planning for IO-PDT in preclinical animal models and eventually in the clinic.

Acknowledgements
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Conflicts of Interest
GS, DB are co-inventors in a Roswell Park patent application for the OSA.

References

1. Ayachit; Utkarsh; The ParaView Guide: A Parallel Visualization Application, Kitware, (2015), ISBN 978-1930934306
2. Python Software Foundation, https://www.python.org/
3. Schroeder, W.; Martin, K.; Lorensen, B., *The Visualization Toolkit* (4th ed.), Kitware, (2006), ISBN 978-1-930934-19-1
4. Cassidy, J; et al.; "High-performance, robustly verified Monte Carlo simulation with FullMonte," J. Biomed. Opt. 23(8) 085001 (2018)
5. Monro, S; et al.; "Transition metal complexes and photodynamic therapy from a tumor-centered approach: challenges, opportunities, and highlights from the development of TLD1433" Chemical Reviews *119* (2) (2019), 797-828 DOI: 10.1021/acs.chemrev.8b00211

> OC079. Oral Communication
Symposium PDT-13 Short Communications on Cancer PDT (M. Lluïsa Sagristá)

STIMULI-RESPONSIVE NANOGELS FOR DUAL PHOTODYNAMIC THERAPY AND CHEMOTHERAPY OF CANCER

Authors: Aimie Rendle[1], Ross Boyle[1], Michael Reithofer[2], Jia Min Chin[1], Huguette Savoie[1]
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1) University of Hull 2) University of Vienna

Background and Objectives

Porphyrins are well known for their photosensitising abilities and hence, their use in photodynamic therapy, however, there are drawbacks to photosensitisers. Some common issues being low wavelength absorptions, poor solubility in biological media, and the poor singlet oxygen quantum yields that arise from this issue. Belali *et al.* demonstrated the use of hydrogels to incorporate porphyrin photosensitisers, using Protoporphyrin IX in a N-isopropyl acrylamide (NIPAM) polymer.[1] NIPAM-based hydrogels are attractive as they have the potential to incorporate a lower critical solution temperature (LCST) above which the physical properties of the hydrogel can change.[2]

Cisplatin is a commonly used platinum(II) chemotherapeutic used in the treatment of prostate, ovarian, and head and neck cancers, amongst others. Whilst showing an over 90% cure rate in the treatment of prostate cancer, cisplatin is well known for its plethora of side effects including neurotoxicity, nephrotoxicity and ototoxicity as well as sever nausea and vomiting. As well as these drawbacks to platinum(II) chemotherapy, there have been increasing reports of platinum-associated chemotherapy-resistance over the past decade.[3]

Results and Discussion

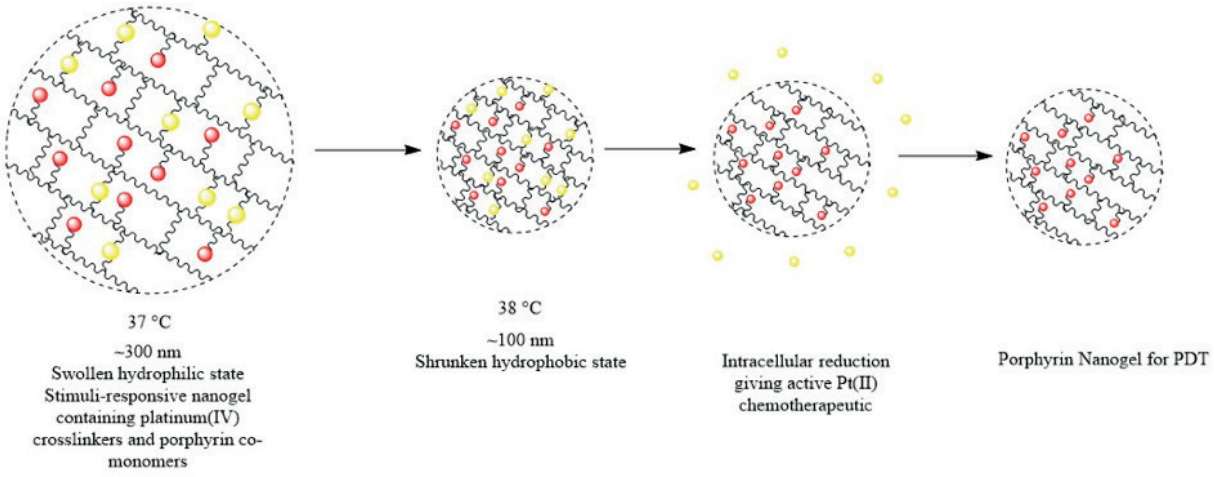
This research focusses on the co-delivery of platinum(II) based chemotherapeutics with porphyrin photosensitisers for PDT. So far, a novel platinum(IV) crosslinker has been synthesised and incorporated into a NIPAM-based nanogel. The platinum(IV) nanogel was shown to have a size of 342 nm in its swollen, hydrophilic state and shrinks to 142 nm when heated above its LCST at 38 °C to give its hydrophobic gel state. The reduction potentials of such nanogels have also been measured to show the viability of a platinum(IV) reduction to platinum(II) by intracellular reducing agents; i.e. ascorbate or alternatively, glutathione.

Two novel hydrogels for use in photodynamic therapy have been synthesised. These include a hydrogel containing Protoporphyrin IX in an adapted synthesis analogous to Blackburn *et al.* whereby PPIX acts as a crosslinker in the hydrogel matrix, we were able to show that upon heating to 37 °C the nanogels reduced in size to 174 nm in their hydrophobic gel state, in comparison to a size of 403 nm in their hydrophilic solution state at 25 °C. In previous literature, Belali *et al.* synthesised PPIX hydrogels, however, although the NIPAM-based hydrogel showed excellent solubility in biological media, the hydrogels showed an LCST of above 40 °C, which is not useful for applications *in vivo*.[1] As well as this, a novel hydrogel containing a cationic water soluble porphyrin monomer has been synthesised. The aim herein is to combine these platinum(IV) crosslinkers and porphyrin monomers into one dual-therapeutic hydrogel.

References

1. S. Belali, H. Savoie, J. M. O'Brien, A. A. Cafolla, B. O'Connell, A. R. Karimi, R. W. Boyle and M. O. Senge, *Biomacromolecules*, 2018, **19**, 1592–1601.
2. S. Mura, J. Nicolas and P. Couvreur, *Nat. Mater.*, 2013, **12**, 991–1003.
3. T. C. Johnstone, K. Suntharalingam and S. J. Lippard, *Chem. Rev.*, 2016, 116, 3436–3486.

Continued


37 °C
~300 nm
Swollen hydrophilic state
Stimuli-responsive nanogel
containing platinum(IV)
crosslinkers and porphyrin co-
monomers
38 °C
~100 nm
Shrunken hydrophobic state
Intracellular reduction
giving active Pt(II)
chemotherapeutic
Porphyrin Nanogel for PDT

SYMPOSIUM COMMUNICATIONS

PLANT PHOTOBIOLOGY

> IL152. Invited Lecture
Symposium PLANT-1 Light Harvesting Complexes (Noam Adir)

LIGHT HARVESTING BY PHOTOSYSTEM I: IN VITRO VS. IN VIVO

Authors: Volha U Chukhutsina[Vrije], Roberta Croce[Vrije]
Presenting Author: Roberta Croce
1) Vrije Universiteit Amsterdam

Photosystem I is a major player in the light reactions of photosynthesis. In higher plants, it consists of a core complex and four external antennae, Lhca1-4 forming the PSI-LHCI supercomplex. The protein and pigment composition, and the spectroscopic properties of this complex are considered to be identical in different plant species. But is this really the case? And is the purify PSI a good representation of the complex *in vivo*? To answer these questions we have performed time-resolved fluorescence measurements on purified PSI complexes and on leaves. The results of four well-studied plant species *Arabidopsis thaliana*, *Zea mays*, *Nicotiana tabacum* and *Hordeum vulgare,* will be presented.

> IL150. Invited Lecture
Symposium PLANT-1 Light Harvesting Complexes (Noam Adir)

SEARCHING FOR PIGMENT CLUSTERS CATALYSING PHOTOPROTECTIVE RESPONSE IN THE ANTENNA SYSTEM OF HIGHER PLANTS

Authors: Luca Dall'Osto[1], Zeno Guardini[1], Roberto Caferri[1], Mauro Bressan[1], Roberto Bassi[1]
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Photosynthetic light harvesting in plants is regulated by a number of mechanisms, including the non-photochemical quenching (NPQ) of excess absorbed light. NPQ modulates the heat dissipation of chlorophyll excited states, thus working as a safeguard in the PSII peripheral antenna. By constructing *Arabidopsis thaliana* plants devoid of specific antenna proteins, namely major trimeric LHCII, monomeric LHCs or both, we found they fulfill different but complementary roles in NPQ, with different quenching sites located in different domains of PSII antenna system. In particular, *Arabidopsis* deletion mutants for the light-harvesting complexes CP29 (*koLhcb4*) are devoid of the fast-activated response of quenching. Catalysis of dissipative reactions requires perception of lumen acidification and interactions between chromophores, either carotenoid, chlorophyll or both. We identified domains involved in quenching by complementing *koLhcb4* plants with sequences deleted in pigment binding or pH sensitive sites. The characterization of transgenic lines demonstrated that the pigment cluster Violaxanthin-*a*603-*a*609 was especially critical for CP29 photoprotective response, indeed quenching was severely affected when the cluster was destroyed. Instead, protonatable residues exposed to the thylakoid lumen were not essential for activation of thermal dissipation *in vivo.* These results are consistent with the model that pH-dependent protein conformational changes, transduced to CP29, alter the coupling strength between chlorophylls *a*603-*a*609 and carotenoid bound to site L2 in this antenna, and catalyze dissipation response.

> IL146. Invited Lecture
Symposium PLANT-1 Light Harvesting Complexes (Noam Adir)

DISTRIBUTING THE ENERGY BETWEEN PHOTOSYSTEM I AND PHOTOSYSTEM II

Authors: Emilie Wientjes[1], Terry Bricker[2], Roberta Croce[3], Herbert van Amerongen[1]
Presenting Author: Emilie Wientjes
1) Laboratory of Biophysics, Wageningen University 2) Department of Biological Sciences, Louisiana State University, USA 3) Life and Biophotonics, VU University Amsterdam, The Netherlands

Introduction

Photosynthesis powers nearly all life on Earth. The absorption of sunlight by pigments in photosystems drives photosynthesis. In the linear electron transport chain Photosystem I (PSI) and Photosystem II (PSII) work in series to extract electrons from water and reduce $NADP^+$. As PSI and PSII work together it is important that the excitation pressure on the two photosystems is balanced. A change in the light spectrum can result in unbalanced excitation of the photosystems. State transitions form the short term acclimation process that redistributes the excitation energy by the movement of light-harvesting complex II (LHCII) from the over-excited photosystem to the light-limited photosystem [1]. When the mobile LHCII is unphosphorylated it associates with PSII, while the phosphorylated forms attaches to PSI [1, 2]. Several open questions remain to be answered about how excitation-energy is distributed between PSI and PSII. It is for instance not clear if LHCII moves between grana and stroma membranes during state transitions or that the change occurs in the grana margins. After long term acclimation to low light plants increase their LHCII level to increase light absorption. How is this LHCII is distributed between PSI and PSII? It is usually assumed to associate with PSII, but is this really the case?

Methods and Results

Ultrafast time-resolved fluorescence was used to investigate the antenna size of PSI in various membrane fractions isolated from the thylakoids of plants. The results show that multiple LHCII can efficiently transfer energy to a single PSI [3]. Not only phosphorylated [4], but also unphosphorylated LHCII can transfer energy to PSI. The change in antenna size of PSI upon state transitions was measured in intact leaves. This change is in agreement with a transfer of ~0.5 LHCII trimer from PSII in state 1 to PSI in state 2. Fluorescence lifetime imaging (FLIM) of chloroplasts in their natural leaf environment [5] and biochemical analysis of the stroma membrane strongly indicates that LHCII moves between grana and stroma during state transitions.

Summary and outlook

While LHCII is classically assumed to be a light harvester for PSII, which in special conditions moves to PSI, more and more evidence is accumulating that indicates that LHCII is also a PSI antenna. Not only in its phosphorylated form, when it forms the digitonin stable PSI-LHCII supercomplex, but also under conditions in which PSI is over-excited and LHCII is not phosphorylated. Future research needs to elucidate how the distribution of this unphosphorylated LHCII between PSI and PSII is regulated.

References

[1] J.F. Allen, BBA, 1098 (1992) 275-335.
[2] X.W. Pan et al, Science, 360 (2018) 1109-1112.
[3] I. Bos et al., BBA, 1858 (2017) 371-378.
[4] E. Wientjes et al., BBA, 1827 (2013) 420-426.
[5] E. Wientjes et al. BBA, 1858 (2017) 259-26

> IL148. Invited Lecture
Symposium PLANT-1 Light Harvesting Complexes (Noam Adir)

TUNING ENERGY TRANSFER EFFICIENCY IN LIGHT HARVESTING ANTENNA OF MARINE CYANOBACTERIA IN RESPONSE TO LIGHT INTENSITY

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Light harvesting in photosynthesis is a remarkable process, taking place under a broad range of environmental conditions. Our approach to study the physical mechanisms by which the harvested energy flow is regulated, was to compare different acclimation states of the same photosynthetic apparatus. We examined *in-vivo*, under ecological relevant conditions, a marine cyanobacteria species that is well adapted to vertical mixing of the water column in the ocean and can acclimate to a broad range of light conditions.

We found that lower light intensity prompts extensive morphological changes. Cells grown under low light were bigger and contained three to four photosynthetic thylakoid membranes, instead of a single membrane observed at medium light intensities. Antennae rods were extended, using additional pigments to better absorb the blue light that penetrates the depth of the water column. In contrast to simple classical energy transfer calculations and to the results reported in vascular plant antenna systems, these longer rods transferred energy faster. Hence, not only that the number of photosynthetic units used in the bacteria is increased, but also the energy transfer efficiency in each photosynthetic unit is enhanced. The fluorescence lifetime and emission spectra dependence on temperature, at the range of 4-300K, measured *in vivo*, suggests that energy transfer efficiencies are tuned by altering the coupling strength of the antennae pigments.

> IL149. Invited Lecture
Symposium PLANT-1 Light Harvesting Complexes (Noam Adir)

PHYCOCYANIN CAN BE SIGNIFICANTLY RED-SHIFTED IN A. MARINA PHYCOBILISOMES

Authors: Noam Adir[1], Shira Bar Zvi[1], Maayan Suissa[1], Avital Lahav[1], Dvir Harris[1], Dariusz M. Niedzwiedzki[2], Robert E. Blankenship[2,3]
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The major light harvesting antenna in all cyanobacterial species is the phycobilisome (PBS). The smallest PBS identified to date is that of the cyanobacterium, *Acaryochloris marina (A. marina),* that is composed of single four-hexamer rod that form quasi-crystalline arrays spanning the cytoplasm between two thylakoid membranes. This organism contains chlorophyll *d*, which has a red-shifted absorption (into the near-IR) when compared to chlorophyll *a.* Past studies suggested that the *A. marina* PBS (AmPBS) contains a single hexamer that contains both phycocyanin (PC) and allophycocyanin (APC), which enables more efficient energy transfer to chlorophyll *d* in Photosystem II. We have studied AmPBS isolated from cells grown under different light regimes by structural, biochemical and spectroscopic methods [1,2]. Spectroscopic and crystallographic analysis of the AmPBS, show that the expression and assembly of different phycocyanin (PC) isoforms can exhibit red-shifted absorption and emission, without the presence of APC. The crystal structure of AmPC revealed additional facets that allow for changes in emission, including the lack of methylation of Asn72 on the β subunits. Ultrafast time-resolved absorption and fluorescence spectroscopies of AmPBS isolated from cells grown under low growth light intensities exhibit similar properties in high (assembled) or low (disassembled) phosphate buffer, indicating that each trimer has the same red-shifted characteristic. Combined spectroscopic and kinetic analysis of this data allowed us to identify spectrally different forms of phycocyanobilins and to propose a minimal model how they may be distributed within the phycobilisome structure. Recently, we have isolated a fraction of AmPC that is further red shifted, exhibiting splitting in its absorption peaks at 653 and 620 nm, similar to APC in the PBS from other species.

Acknowledgments
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References

[1] Structural heterogeneity leads to functional homogeneity in *A. marina* phycocyanin. S. Bar-Zvi, A. Lahav, D. Harris, D.M. Niedzwiedzki, R.E. Blankenship and N. Adir. Biochimica et Biophysica Acta – Bioenergetics 1859, 544-553 (2018).
[2] Excitation energy migration pathways between multiple phycocyanin populations in *A. marina* phycobilisomes D.M. Niedzwiedzki, S. Bar-Zvi, R.E. Blankenship and N. Adir. BBA Bioenergetics 1860, 286-296 (2019).

> IL151. Invited Lecture
Symposium PLANT-1 Light Harvesting Complexes (Noam Adir)

PHYCOBILISOMES' SECRET LIFE

Authors: Michal Gwizdala[1,2], Tjaart P.J. Krüger[1]
Presenting Author: Michal Gwizdala
1) University of Pretoria 2) Vrije Universiteit Amsterdam

In many strains of cyanobacteria and algae, phycobilisomes (PBs) absorb light and transfer excitations to the photosynthetic reaction centres, where excitation energy is converted into chemical energy. In PBs from *Synechocystis* PCC6803, nearly 400 identical pigments, called phycocyanobilins, are covalently bound to the protein subunits that form a hand-like structure. Due to different pigment-protein interactions, these subunits differ in their optical properties, making PBs efficient in transferring energy from the distal parts of the complex (the so-called rods) to the central core and to the photosystems. The optical properties of the PB pigments have been studied for nearly 200 years and are now considered well-explored. But are they really?

Recently, single molecule spectroscopy (SMS) has revealed rich spectroscopic dynamics of PBs [1]. For the first time in any SMS study, we performed our measurements using physiologically relevant light intensities and discovered a novel type of energy dissipation mechanism in intact isolated PBs. This mechanism is directly light activated and does not require interactions with other proteins. In fact, switching between energy dissipative and light-harvesting states involves a conformational change in the protein scaffold and likely a configurational change in the pigment structure.

We have also explored the main photoprotective mechanism of cyanobacteria, involving the orange carotenoid protein (OCP), at the SMS level [2]. By controlling in real-time the interaction between the two key players – individual PBs and single OCPs – we revealed an intermediate state of quenching signifying the docking and undocking of OCP on a PB complex. In this intermediate, partly quenched state some of the rods temporarily disconnect from the PBs' core and a hidden OCP-induced red state is exposed. These states possibly reveal crucial mechanistic details of energy quenching.

Interestingly, not all hidden states of PBs are quenched or partly quenched. The isolated rods of PBs can assume two different states, both of which are possibly involved in energy transfer to the photosystems [3]. While one of these states fits the well-established model of energy transfer in PB, the other unusual state is characterized by red-shifted emission and most likely is involved in energy transfer directly to photosystem I. Switching between these states also involves a conformational change. This work allowed us to redefine the function of linker proteins in PBs, by showing that these linker proteins only stabilise an intrinsic state that is accessible by the pigment-proteins in the absence of the linker proteins.

References

[1] Gwizdala, M., Berera, R., Kirilovsky, D., van Grondelle, R., Krüger, T.P.J., Controlling light harvesting with light, *J. Am. Chem. Soc.*, Vol. 138(36):11616-11622, 2016
[2] Gwizdala, M., Botha, J., Wilson, A., Kirilovsky, D., van Grondelle, R., Krüger, T.P.J., Switching an individual phycobilisome off and on, *J. Phys. Chem. Lett.*, Vol9(9):2426-2432, 2018
[3] Gwizdala, M., Krüger, T.P.J., Wahadoszamen, Md., Gruber, J.M., van Grondelle, R., Phycocyanin: one complex, two states, two functions, *J. Phys. Chem. Lett.,* Vol9(6):1365-1371, 2018

> IL147. Invited Lecture
Symposium PLANT-1 Light Harvesting Complexes (Noam Adir)

CONSTRUCTION OF GENETICALLY ENCODED FLUORESCENT TEMPERATURE SENSOR DERIVED FROM THE PHOTOACTIVE ORANGE CAROTENOID PROTEIN

Authors: Eugene G. Maksimov[1], Igor A. Yaroshevich[1], Georgy V. Tsoraev[1], Nikolai N. Sluchanko[1, 2], Ekaterina A. Slutskaya[3], Thomas Friedrich[4], Alexey V. Stepanov[3]
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Photoactive proteins occupy a crucial position in science due to the potential to serve as scaffolds for the construction of novel sensors, signalling cascades and light-triggered photoswitches for fundamental and applied research. The cyanobacterial Orange Carotenoid Protein (OCP) is one of the examples of such photoactive proteins within a rapidly developing field of science. Initial investigations in this area have been recently reported in Nature, PNAS, Science and other top-rated journals. Until now, the mechanisms of OCP functioning were investigated in the context of its physiological role as a quencher of excitation energy, which protects photosynthetic antennae. But the actual mechanism of energy dissipation by OCP is poorly understood yet. The problem is that cyanobacterial antennae emit at wavelengths (~ 660-680 nm) where absorption of OCP in any of its spectroscopically distinct forms is very low. Thus, the overlap between the emission of the excitation energy donor and the absorption of the energy acceptor is small and cannot afford a sufficiently high energy transfer rate to compete with the energy transfer from the antenna to the chlorophylls of the photosystems. Also, the structure of the OCP-antenna complex is unknown due to complexity of huge antennas consisting of hundreds of pigments. So, any kind of estimations of excitation energy transfer in such complex systems are speculative and full of assumptions. Thus, it is reasonable to assume that a simple (binary) model of antenna-quencher may be useful for the study of energy dissipation and photoprotection.

A few years ago, we realized that the carotenoid in OCP acts as a polyspecific quencher, not only for cyanobacterial phycobilisomes, since it is able to quench fluorescence of (non)-covalently bound organic dyes and, notably, the intrinsic fluorescence of Trp. In principle, such a reduction of excited states upon the interaction with OCP is similar to quenching of photosynthetic antennas. Considering the fact that OCP can be used as a molecular thermometer, since its photocycling strongly depends on temperature, these observations inspired us to construct a genetically encoded temperature sensor, with a fluorescence readout. In this work we were focused on explanation of excitation energy transfer which occurs between the chromophore of fluorescent proteins and the carotenoid of OCP in a single chimeric construction. We report that such artificial systems, which mimic donor-acceptor interactions in the native OCP-antenna complexes, are photoactive and could be used for temperature measurements in biological systems. Future directions of OCP-based sensor improvement will be discussed.

> OC081. Oral Communication
Symposium PLANT-1 Light Harvesting Complexes (Noam Adir)

REGULATION OF THE PHOTOSYNTHETIC LIGHT HARVESTING REQUIRES ONLY THE PROTON GRADIENT AND THE MAJOR LHCII ANTENNA COMPLEX

Authors: Alexander Ruban[1]
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Plants are subject to dramatic fluctuations in the intensity of sunlight throughout the day. When the photosynthetic machinery is exposed to high light, photons are absorbed in excess, potentially leading to oxidative damage of the delicate membrane components of photosynthesis. A physiological mechanism of photoprotection called NPQ, is the fastest, response carried out in the thylakoid membranes to dissipate harmlessly the energy in excess. There is still intense debate about the key molecular details of this mechanism. Here, we show that that quickly reversible component of NPQ, qE, is present in thylakoids largely enriched in only the major trimeric light-harvesting complex (LHCII) in the complete absence of all minor LHC complexes and with strongly reduced amounts of photosystem core proteins. This fast and reversible quenching depends upon thylakoid lumen acidification and involves aggregation of LHCII. Enhancing ΔpH amplifies the extent of the quenching and restores qE in the membranes lacking PsbS protein. The carotenoid zeaxanthin modulates the kinetics and amount of quenching as in wild-type plants, accelerating the formation and delaying the recovery in agreement with the allosteric model of NPQ. These findings show that the nature evolved the photosynthetic light harvesting of plants with the self-regulatory properties, where the major LHCII complex is capable of reversible switching between efficient harvesting and photoprotective states.

> OC080. Oral Communication
Symposium PLANT-1 Light Harvesting Complexes (Noam Adir)

MICRO-SCATTERING SPECTROSCOPY COMBINED WITH PAM CHLOROPHYLL FLUOMETRY FOR SIMULTANEOUS INVESTIGATION OF OPTICAL PROPERTIES AND PHOTOSYNTHESIS

Authors: Johannes Wilhelm Goessling[1], Wiliam Peter Wardley[1], Miguel Castillo[1], Martín López-García[1]
Presenting Author: Johannes Wilhelm Goessling
1) International Iberian Nanotechnology Laboratory

Introduction
Many phototrophic organisms exhibit highly ordered cellular features at the micro/nano-scale that resemble photonic structures. Prominent examples are thylakoid membranes of plants, which stack in layers of a varying number according to the lighting conditions, or perforated cell walls of some algae that can guide and redistribute light over the cell. To evaluate whether such structures can also facilitate light harvesting for efficient photosynthesis has been, to date, complicated as the photonic properties and photosynthetic activities could not be measured simultaneously. We have developed an optical microscope that combines pulse amplitude modulated (PAM) chlorophyll fluometry with Fourier image microscatterometry, allowing for simultaneous observation of photonic properties and photosynthesis with microscopic and millisecond-temporal resolution.

Results and Discussion
Although photonic structures are widespread in phototrophic organisms, little is known about their potential implications upon the photo-physiology of organisms. We studied photonic structures and their potential implications upon photosynthesis in different phototrophic clades, including vascular plants, mosses, and photosynthetic protists. We can show that photonic structures alter the photonic environment in different ways. For instance, specialized thylakoid stacks in shade-dwelling *Begonia sp.* (Fig. 1) can enhance light absorption in the green spectral range that is more available in the understory of tropical rain forests [1], mosses of the genus *Schistostega sp.* have evolved spherical cells to enhance incident light propagating at the entrances of caves and abandoned mines, and diatoms living in aquatic environments produce photonic crystal like silicate cell walls that modulate the light field inside and around the cell [2].

Conclusion
We can demonstrate that biological photonic structures are used in different ways to advance light harvesting of phototrophic organisms. We conclude that such structures influence the photonic environment in compartments of the cell where photosynthesis takes place. We propose that such structures can also inspire the development of natural inspired photonic application.

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Conflicts of Interest
The authors declare that they have no conflict of interest.

References

[1] M. Jacobs, M. Lopez-Garcia, O.-P. Phrathep, T. Lawson, R. Oulton, and H. M. Whitney, “Photonic multilayer structure of Begonia chloroplasts enhances photosynthetic efficiency” Nat. Plants 2, 16162 (2016).
[2] J. W. Goessling, S. Frankenbach, L. Ribeiro, J. Serôdio, and M. Kühl, “ Modulation of the light field related to valve optical properties of raphid diatoms: implications for niche differentiation in the microphytobenthos,” MEPS (588), 29–42 (2018).

> IL154. Invited Lecture
Symposium PLANT-2 Photosystem II and Oxygen Evolution (Richard Debus)

MECHANISM OF PHOTOSYNTHETIC WATER OXIDATION

Authors: Jian-Ren Shen[Okaya]
Presenting Author: Jian-Ren Shen
1) Okayama University

Photosynthetic water oxidation is catalyzed by a Mn_4CaO_5-cluster embedded in the protein matrix of Photosystem II (PSII). The water oxidation proceeds through four sequential steps via the S*i*-state cycle (S_i, *i* = 0-4). We have solved the structure of the Mn_4CaO_5-cluster by both synchrotron radiation X-rays[1] and femtosecond X-ray free electron lasers (XFEL)[2] at atomic resolutions. These studies revealed a "distorted" chair form of the catalytic center and detailed arrangement of each atoms, inter-atomic distances within the Mn_4CaO_5-cluster, in its dark-stable S_1-state. In order to fully uncover the reaction mechanism of water oxidation, it is necessary to solve the structures of the catalyst in its intermediate S-states. To this end, we used a pump-probe approach with a combination of "small" PSII crystals and serial femtosecond X-ray crystallography (SFX) using the femtosecond XFELs, to solve the structures of the intermediate S-states. We have reported the structure of 2-flashes induced S_3-state[3] in which, a new oxygen was found to be inserted in a position close to O5, a unique oxo-bridged oxygen already present in the S_1-state. Our results suggested the formation of O=O bond between O5 and O6. Due to the limited resolution, however, there are still uncertainties regarding the distance between O5 and O6, and thus the exact mechanism of O=O bond formation was still unclear. We have improved the resolution of the intermediate S_3-state structure, and also solved the 1-flash induced S_2-state structure. Based on these results, the molecular mechanism for O=O bond formation has now become clear.

Acknowledgments
I thank all of the collaborators who are involved in the work presented in this talk but not listed here due to the limited space.

References

1. Umena Y., Kawakami K., Shen J.-R., Kamiya N. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* **473**, 55-60, (2011).
2. Suga, M. *et al.* Native structure of photosystem II at 1.95 Å resolution viewed by femtosecond X-ray pulses. *Nature* **517**, 99-103, (2015).
3. Suga, M. *et al*. Light-induced structural changes and the site of O=O bond formation in PSII caught by XFEL. *Nature* **543**, 131-135, (2017).

> IL156. Invited Lecture
Symposium PLANT-2 Photosystem II and Oxygen Evolution (Richard Debus)

SPECTROSCOPIC AND SCATTERING STUDIES OF PHOTOSYSTEM II UTILIZING FS X-RAY PULSES

Authors: Jan Kern[1], Mohamed Ibrahim[2], Franklin Fuller[3], Markus Kubin[4], Louise Lassalle[5], Aaron Brewster[1], Sheraz Gul[1], Roberto Alonso-Mori[3], Casper de Lichtenberg[5], Mun Hon Cheah[5], Iris Young[1], Rana Hussein[2], Rolf Mitzner[4], Philipe Wernet[4], Johannes Messinger[5], Athina Zouni[2], Nicholas Sauter[1], Uwe Bergmann[2], Vittal Yachandra[1], Junko Yano[1]
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Introduction

The recent availability of fs X-ray pulses from XFELs makes it possible to probe the active site of metalloenzymes at room temperature in a time resolved manner without the problems of radiation damage. Conducting such studies, nevertheless is hindered by several technical bottlenecks. These include very limited experimental time available at the few X-ray laser sources currently operating, often very high sample consumption rates and challenges in data collection and processing. When over coming these bottlenecks XFEL pulses are an ideal tool to study photosynthetic systems due to the possibility to conduct optical laser pumping-x-ray probe experiments. Photosystem II (PSII) is a membrane instrinsic protein complex that catalyzes the light driven oxidation of water to molecular oxygen [1]. To better understand the catalytic mechanism of PSII we were utilizing fs X-ray diffraction and X-ray emission as well as X-ray absorption spectroscopy at the Mn K- and L-edges.

Results and Discussion

We will present our current progress in XFEL studies of PSII. Recent results include first undamaged Mn L-edge spectra of PSII in two different illumination states [2], kinetic measurements of Mn oxidation state changes at room temperature using Mn Kb emission spectroscopy and time resolved crystallographic determination of the structure of several intermediates in the catalytic cycle of water oxidation [3,4]. We determined the structure for the catalytic site in PSII, the Mn4CaO5 cluster at around 2.0 A resolution at room temperature in all four stable intermediate states (S states). Especially we could demonstrate the location of an additional Oxygen (Ox) bound to the Mn_4CaO_5 cluster in PSII in the S_3 state and track the steps involved in the formation of that state in time resolved measurements [4]. The observed structural changes and the implication for the mechanism of water oxidation in PSII will be discussed.

References

[1] J. Yano and V. Yachandra, Chem. Rev. **114**, 4175 (2014)
[2] M. Kubin *et al.*, Struct. Dyn. **4**, 054307 (2017)
[3] I. D. Young *et al.*, Nature **540**, 453 (2016)
[4] J. Kern *et al.*, Nature **563**, 421 (2018)

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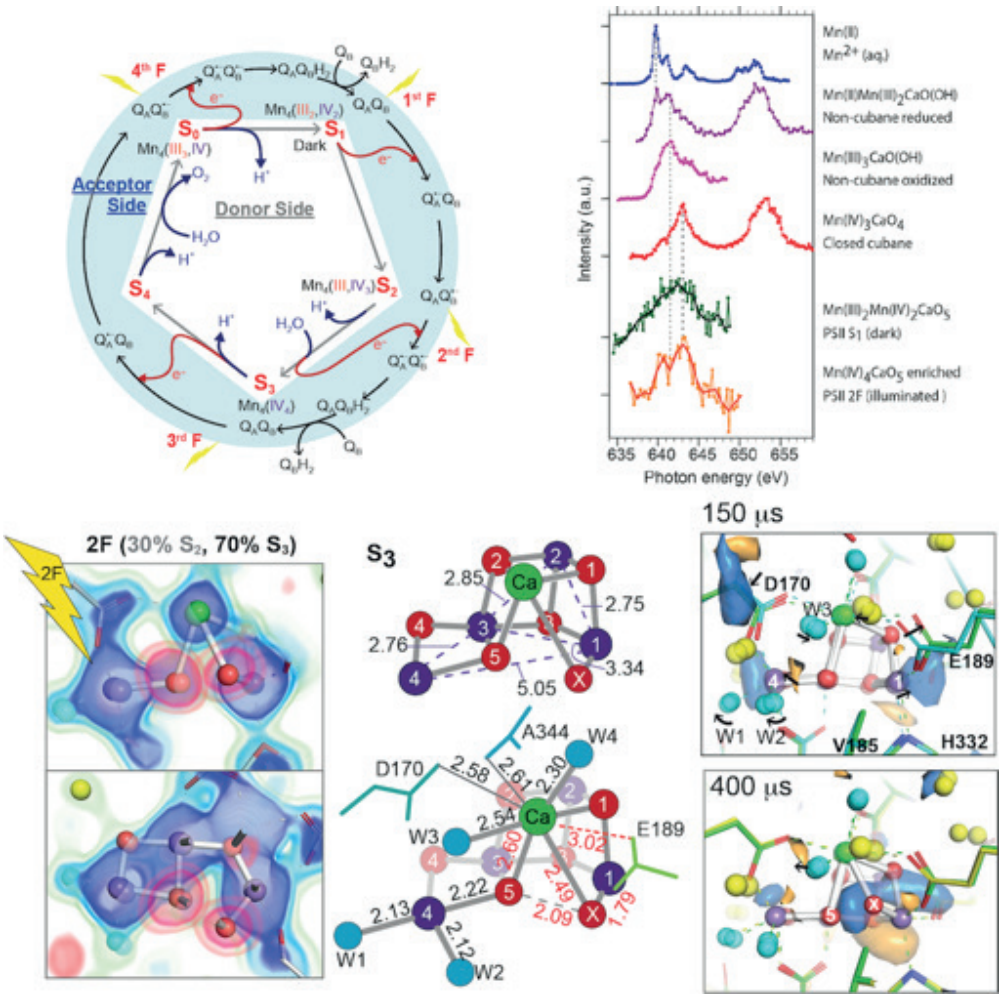

Figure Legend: Reaction cycle of PSII (top left) and Mn L-edge spectra of model compounds and PSII (right). Electron density obtained at RT for the double illuminated state (bottom left) together with structural models and difference electron density obtained for two time points in the transition from the S_2 to the S_3 state (bottom right).

> IL153. Invited Lecture
Symposium PLANT-2 Photosystem II and Oxygen Evolution (Richard Debus)

QUANTUM CHEMICAL STUDIES OF REDOX ENZYMES

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Presenting Author: Per Siegbahn
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Traditionally, enzyme mechanisms have been studied mainly by X-ray diffraction and spectroscopy. Since about 10 years, a complement to those studies in terms of theoretical modeling has also become an essential part of the studies. A major reason theoretical studies are essential is that many of the states involved in mechanisms are too short-lived to be observed experimentally. These cases concern, in particular, the most interesting and decisive parts of the mechanism, in which the rate determining steps occur. For water oxidation in PSII, a mechanism was suggested in 2006, which has stood all tests by experiments the past decade. In short, the formation of the critical O-O bond was suggested between a bridging oxo group and a terminal oxygen radical in the OEC. Detailed structures for all the S-states were given in 2008. In 2011, the first high-resolution X-ray structure confirmed most of the predictions made. More recently, a mechanism for water insertion in the S_2 to S_3 transition was made, and detailed possible structures of S_3 were investigated in light of recent XFEL structures. Many other redox enzymes have also successfully been studied using the same methodology

> IL158. Invited Lecture
Symposium PLANT-2 Photosystem II and Oxygen Evolution (Richard Debus)

A NEW INTERMEDIATE IN THE ACTIVATION OF NATURE'S WATER SPLITTING COFACTOR

Authors: Nick Cox[1]
Presenting Author: Nick Cox
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Recently, the last metastable intermediate (S_3 state) of nature's water splitting cofactor, the Mn_4O_5Ca cluster of Photosystem II (PSII) was characterized by high-field (W-band) EPR spectroscopy and serial femtosecond X-ray crystallography (SFX) (1-3). These data are only consistent with an all octahedral Mn^{IV} complex, which has an additional water derived molecule bound to Mn1. It is this activated form of the cofactor that goes on to form the O-O bond. An important question that has yet to be resolved is how cofactor activation occurs during the S_2-S_3 transition. Here we report a new class of intermediate (S_3\') in which cofactor oxidation has occurred without water insertion. This intermediate can be trapped in significant fraction of centers (>50%) in: (a) PSII in which Ca^{2+} is exchanged with Sr^{2+}; the Mn_4SrO_5 cofactor remains active but has a significantly lower rate of O_2 evolution than Mn_4CaO_5 (4); and (b) PSII with 3% v/v methanol added; methanol is thought to act as a substrate water analogue (5). The S_3\' EPR signal is significantly broader than in the wild-type (2.5 T vs. 1.5 T). It is this increase in spectral width that is indicative of the cofactor still containing a five-coordinated Mn ion, as seen in the preceding S_2 state. Magnetic double resonance data support these findings revealing the electronic connectivity of the S_3\' cofactor is similar to the high spin form of the preceding S_2 state which contains a cuboidal Mn_3O_4Ca unit tethered to an external, five coordinate Mn ion (Mn4). These results demonstrate that cofactor oxidation initiates, and can be decoupled from, water molecule insertion. The interaction of ammonia, another water analogue, with the cofactor in the S_3 state is also discussed (6,7).

References

1. *Cox, N.; Retegan, M.; Neese, F.; Pantazis, D. A.; Boussac, A.; Lubitz, W.* Electronic structure of the oxygen-evolving complex in photosystem II prior to O-O bond formation. *Science* 345(6198):804-808
2. Suga M, et al. (2015) Native structure of photosystem II at 1.95 A resolution viewed by femtosecond X-ray pulses. Nature 517(7532):99-103.
3. Kern J*, et al.* (2018) Structures of the intermediates of Kok's photosynthetic water oxidation clock *Nature* 563:412-425.
4. Boussac A*, et al.* (2004) Biosynthetic Ca^{2+}/Sr^{2+} exchange in the photosystem II oxygen-evolving enzyme of *Thermosynechococcus elongatus*. *J. Biol. Chem.* 279(22):22809-22819.
5. Oyala PH*, et al.* (2014) Pulse Electron Paramagnetic Resonance Studies of the Interaction of Methanol with the S_2 State of the Mn_4O_5Ca Cluster of Photosystem II. *Biochemistry* 53(50):7914-7928.
6. Pérez Navarro, M.; Ames, W. M.; Nilsson, H.; Lohmiller, T.; Pantazis, D. A.; Rapatskiy, L.; Nowaczyk, M. M.; Neese, F.; Boussac, A.; Messinger, J.; Lubitz, W.; Cox, N. (2013) Ammonia binding to the oxygen-evolving complex of photosystem II identifies the solvent-exchangeable oxygen bridge the manganese tetramer. *Proc. Natl. Acad. Sci. U.S.A* 110(39):15561-15566.
7. Oyala PH, Stich TA, Debus RJ, & Britt RD (2015) Ammonia Binds to the Dangler Manganese of the Photosystem II Oxygen-Evolving Complex. *J. Am. Chem. Soc.* 137(27):8829-8837.

> IL155. Invited Lecture
Symposium PLANT-2 Photosystem II and Oxygen Evolution (Richard Debus)

SUBSTRATES OF PHOTOSYNTHETIC WATER OXIDATION

Authors: Mun Hon Cheah[1], Casper de Lichtenberg[1,2], Johannes Messinger[1,2]
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Vast majority of life on Earth is sustained by solar energy captured during photosynthesis in cyanobacteria, alga and plants. Solar energy is stored in the chemical bonds of carbohydrates and other molecules by reducing CO_2 with electrons derived from water oxidation reaction that is catalyzed exclusively by photosystem II (PSII). Catalysis of water oxidation to molecular oxygen is performed at the Mn_4CaO_5 cluster within the oxygen evolving complex (OEC) of PSII. Recent crystal structures of PSII in all semi-stable intermediate states of the catalytic cycle, coupled with spectroscopy and DFT calculations are stimulating rigorous discussions about the detail molecular mechanism of water oxidation. Several plausible mechanisms are under debate, mainly because the binding site of the two substrate water molecules at the Mn_4CaO_5 cluster are disputed.

Substrate exchange kinetics studies of PSII by time resolved membrane inlet mass spectrometry (MIMS) is the only experimental method currently available that gives direct information about substrate water binding to the OEC. In these experiments, PSII is first poised at a specific intermediate state (S-state) of the OEC by light flashes followed $H_2{}^{18}O$ injection. PSII is incubated with labelled ^{18}O water for a specified time before a rapid flash train to complete single turnover. The exchange rate of unlabeled substrate water with the ^{18}O-labelled bulk water is derived by monitoring the isotopic composition of evolved O_2 at m/z 34 and 36 as function of incubation time. Two distinct substrate exchange rates that vary independently from each other with S-state, pH and point mutations are observed. This presentation will review the most important findings in the exchange kinetics and present new data on the substrate exchange in the open and closed conformers of the S_2 state in Ca-PSII and Sr-PSII core complexes of *T. elongatus*. Implications for the substrate exchange mechanism, possible binding sites and water oxidation mechanism will be discussed.

> IL157. Invited Lecture
Symposium PLANT-2 Photosystem II and Oxygen Evolution (Richard Debus)

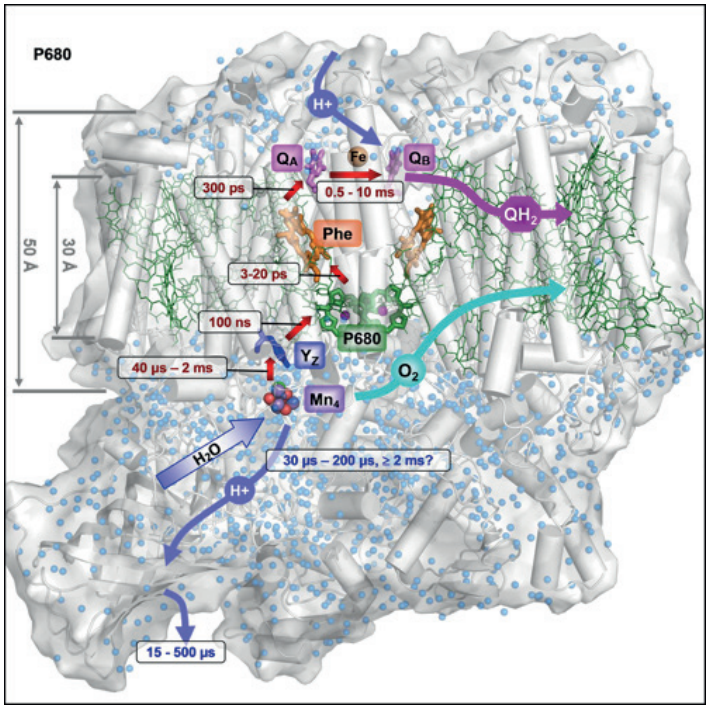
MUCH MORE THAN JUST ELECTRON TRANSFER: PHOTOSYNTHETIC WATER OXIDATION INVESTIGATED BY TIME-RESOLVED INFRARED SPECTROSCOPY

Authors: Philipp Simon[1], Matthias Schönborn[1], Sarah Mäusle[1], Paul Greife[1], Rick Debus[2], Robert Burnap[3], Holger Dau[1]
Presenting Author: Holger Dau
1) Free University Berlin 2) University of California, Riverside 3) Oklahoma State University

Light-driven water oxidation (and the associated oxygen evolution) of cyanobacteria and plants has shaped the Earth\'s biosphere and atmosphere. A huge cofactor-protein complex called photosystem II (PSII, see Figure 1) facilitates this process. Exciting progress in protein crystallography with femtosecond X-ray pulses has started to provide an increasingly detailed picture. Recently nuclear coordinates at currently about 2 Å resolution have been reported not only for the dark-stable resting state of PSII, but also for several intermediate states of the reaction cycle. So far, no major structural changes of the protein conformation were detected, but subtle movements of the oxygen atoms of water molecules. We believe that changing H-bonds and protonation states are likely to play a crucial mechanistic role in light driven water oxidation, which cannot easily be tracked by X-ray crystallography. To address these changes by time-resolved infrared spectroscopy, we have developed new experimental technology and investigated previously hidden events.

PSII particles from spinach and cyanobacteria were driven by nanosecond laser pulses through the water oxidation cycle. In an FTIR step-scan experiment, we monitored the transitions between the four semi-stable states of the water oxidation cycle (S0 to S3) with a time resolution of 10 µs covering the range of about 1000 to 1800 cm^{-1}. At selected wavenumbers, IR transients were recorded with 50 ns time resolution using tunable QCLs (QCL, quantum cascade laser) for plant and cyanobacterial PSII, the latter including investigation of site-specific genetic PSII variants. Specifically, the \'terra incognita\' of the events that precede electron transfer in the S_2-S_3 and the oxygen-evolving S_3-S_0 transition are discussed.

Figure 1. Light-driven processes in PSII (modified from H. Dau, I. Zaharieva, M. Haumann (2012) Curr. Opinion Chem. Biol. 16, 3-10).



> P093. Poster

Symposium PLANT-2 Photosystem II and Oxygen Evolution (Richard Debus)

QUANTITATIVE ASSESSMENT OF THE HIGH-LIGHT TOLERANCE IN PLANTS WITH AN IMPAIRED PHOTOSYSTEM II DONOR SIDE

Authors: Sam Wilson[1], Alexander Ruban[1]
Presenting Author: Sam Wilson
1) School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London, E1 4NS, United Kingdom

Photoinhibition is the light-induced downregulation of photosynthetic efficiency, the primary target of which is photosystem II (PSII). Currently, there is no clear consensus on the exact mechanism of this process. However, it is clear that inhibition can occur through limitations on both the acceptor- and donor-side of PSII. The former mechanism is caused by electron transport limitations at the PSII acceptor side. Whilst, the latter mechanism relies on disruption of the oxygen-evolving complex (OEC). As a consequence of both of these mechanisms, the PSII reaction centre (RC) and the surrounding environment are irreversibly damaged. Using a novel chlorophyll fluorescence methodology, RC photoinactivation can be sensitively measured and quantified alongside photoprotection *in vivo*. This is achieved through estimation of the redox state of Q_A, using the parameter of photochemical quenching in the dark (qPd). This study shows that through the use of PSII donor-side inhibitors, such as UV-B and Cd^{2+}, there is a steeper gradient of photoinactivation in the systems with a weakened donor side, independent of the level of NPQ attained. This is coupled with a concomitant decline in the light tolerance of PSII. The native light tolerance is partially restored upon use of 1,5-diphenylcarbazide (DPC), a PSII electron donor, allowing for the balance between the inhibitory pathways to be sensitively quantified. Thus, this study confirms that the impact of donor-side inhibition can be detected alongside acceptor-side photoinhibition using the qPd parameter, and confirms qPd as a valid, sensitive and unambiguous parameter to sensitively quantify the onset of photoinhibition through both acceptor- or donor-side mechanisms.

> IL159. Invited Lecture
Symposium PLANT-3 Photoprotection in Photosynthetic Organisms (Diana Kirilovsky)

THE MECHANISM OF THE OCP-FRP INTERACTION REGULATING PHOTOPROTECTION IN CYANOBACTERIA

Authors: Nikolai Sluchanko[1,2], Yury Slonimskiy[1,3], Marcus Moldenhauer[4], Thomas Friedrich[4], Eugene Maksimov[1,2]
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Photosynthetic organisms need to adapt to changing levels of insolation by adjusting the efficiency of photosynthesis and protecting themselves in high light conditions when the risk of reactive oxygen species formation threatens the integrity of the cells. The cyanobacterial photoprotection system is dictated by their water-soluble antenna complexes, phycobilisomes (PBs), and is based on the interplay between the Orange Carotenoid Protein (OCP) and the Fluorescence Recovery Protein (FRP)[1]. OCP is a two-domain photoactive carotenoprotein which, under blue-green light illumination, reversibly transforms from OCP^O into a PBs-quenching OCP^R form. FRP preferentially binds to OCP^R and accelerates its OCP^R-OCP^O relaxation to inhibit the OCP-mediated photoprotection[2], but the molecular details of this dynamic interaction remained unclear. Noticing a difference in levels of homology between FRP paralogs (≤50% sequence identity) and OCP paralogs (~80-85% sequence identity), we studied the interaction between *Synechocystis* OCP (SynOCP) and selected FRP homologs[3]. The structural analysis confirmed the equivalence of the dimeric conformations of the low-homology FRP variants as well as the possibility of their interaction with SynOCP. However, this functional interaction showed remarkable differences, in particular, in the ability of the FRP homologs to form 2:1 and 1:1 complexes with SynOCP. We suggest that these complexes correspond to intermediary steps of the FRP-OCP interaction, in line with the previously proposed FRP monomerization in the course of its binding to OCP. This was tested using the unique FRP mutants representing constantly monomeric and dimeric forms, which showed that the monomeric FRP variant is inefficient in OCP binding, in contrast to the fixed FRP dimer, which was fully active. Disulfide trapping and chemical crosslinking revealed that FRP binds via its head domain to the C-terminal domain of OCP around the binding site for the N-terminal extension and helped identify complexes with 1:1, 2:1, and 2:2 stoichiometries[4]. Structural analysis in solution allowed us to model FRP-OCP complexes with different stoichiometries supported by the surface conservation and electrostatics analyses of proteins and to tentatively propose the dissociative mechanism regulating high light tolerance in cyanobacteria, which is based on FRP monomerization.

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References

1 Kirilovsky, D. & Kerfeld, C. A. *Nature plants* **2**, 16180 (2016).
2 Sluchanko, N. N. *et al. Biochim Biophys Acta* **1858**, 1-11 (2017).
3 Slonimskiy, Y. B. *et al. Biochim Biophys Acta* **1859**, 382-393 (2018).
4 Sluchanko, N. N. *et al.* OCP-FRP protein complex topologies suggest a mechanism for controlling high light tolerance in cyanobacteria. *Nat Commun* **9**, 3869 (2018).

> IL160. Invited Lecture
Symposium PLANT-3 Photoprotection in Photosynthetic Organisms (Diana Kirilovsky)

PHOTOPROTECTION IN DIATOMS

Authors: Claudia Büchel[1]
Presenting Author: Claudia Büchel
1) Goethe University Frankfurt, Institute of Molecular Biosciences

Diatoms are unicellular algae that contribute with about 25% to the yearly biomass production. Their ecological success is partly based on their ability to perfectly balance efficient light harvesting and photoprotection, whereby they contain higher numbers of light harvesting proteins than vascular plants for these purposes. Due to the binding of the carotenoid fucoxanthin besides Chl c and Chl a, the proteins are called fucoxanthin-chlorophyll proteins (FCP). The number of FCP complexes, their subunit composition and their interactions in the thylakoid membranes remain elusive.

In all group of diatoms several different Lhcx proteins are present. For centric diatoms it could be shown that Lhcx1 is a subunit of the major trimeric FCPa complex (Beer et al. 2006) and that it is involved in photoprotection. Knock-down of Lhcx1 resulted in a reduced ability for the energy dependent (qE) part of non-photochemical quenching, which is a photoprotection mechanism where excess energy is converted to heat at the expense of fluorescence (Ghazaryan et al. 2016).

We used the recently available genome sequence to analyze the genes for putative light harvesting proteins in the centric diatom *Cyclotella meneghiniana*, and to elucidate the FCP complex composition using mass spectrometry. We analyzed two pools of FCP complexes that were trimeric (FCPa) and nonameric (FCPb). FCPa was composed of four different trimeric sub-types. Two different nonameric FCPb complexes were present. All were distinguished by their polypeptide composition and partly by pigmentation. Using a milder purification method, two fractions composed of different FCP complexes were isolated: Band A was enriched in FCPs incorporating Lhcx1, such as the newly identified nonameric FCPb2 and the major trimeric FCPa4 complex. Band B contained mainly FCPs that were devoid of Lhcx1. Both fractions also included small amounts of trimeric FCPa complexes with the centric diatom-specific Lhcx protein, Lhcx6_1, as subunit. The quenching ability had been shown to depend on the Lhcx1 content (Gundermann and Büchel, 2008). Thus, the Lhcx1 containing complexes in Band A should be involved in qE, whereas FCPs of Band B then constitute the basic light harvesting antenna. Whereas the Lhcx1 content depends strongly on the illumination condition, Lhcx6_1 seems to be more constitutively present. Also Lhcx6_1 functions in photoprotection as demonstrated using knock-down mutants. The consequences of this arrangement of FCPs around the photosystems and the distribution of protective subunits will be discussed.

References

Beer A, Gundermann K, Beckmann J, Büchel C. Biochemistry 45 (43), 13046-13053 (2006)
Gundermann K, Büchel C. Photosynth. Res. 95 (2-3), 229-235 (2008)
Ghazaryan A, Akhtar P, Garab G, Lambrev PH, Büchel C. Biochim. Biophys. Acta 1857 (9), 1373-1379 (2016)

> IL164. Invited Lecture
Symposium PLANT-3 Photoprotection in Photosynthetic Organisms (Diana Kirilovsky)

ALGAL PHOTOPROTECTION: MECHANISM AND SIGNAL TRANSDUCTION

Authors: Jun Minagawa[1], Yusuke Aihara[1], Ryutaro Tokutsu[1], Eunchul Kim[1], Kotaro Kosuge[1], Konomi Fujimura-Kamada[1], Tomohito Yamasaki[2], Seiji Akimoto[3], Yoshifumi Ueno[3], Makio Yokono[4]
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Although light is essential for photosynthesis, amounts of light that exceed an organism's assimilation capacity can cause serious damage. Photosynthetic organisms minimize such potential harm via protection mechanisms collectively referred to as non-photochemical quenching (NPQ). One of the NPQ mechanisms is readily activated under high-light (HL) conditions and dissipates excess energy as heat (aka qE-quenching). LHCSR3 is associated with PSII to form the PSII-LHCII-LHCSR3 supercomplex when the green alga *Chlamydomonas reinhardtii* is grown in high light and transforms it into an energy-dissipative form upon lumenal acidification[1,2]. LHCSR1 mediates excitation energy transfer from LHCII to PSI[3], as well as helps to dissipate excess energy in LHCII, which also causes fluorescence quenching. *LHCSR3* is expressed when *Chlamydomonas* cells are grown in high light. For the induction of *LHCSR3* gene, blue-light perception by a blue-light photoreceptor phototropin as well as photosynthetic activity is required[4], although details of its signal transduction pathway have been elusive. To clarify the signal transduction pathway, we identified two loci *det1* and *ddb1* by isolating *phot* suppressor mutants, which exhibited high NPQ phenotype[5]. Using a yeast two-hybrid analysis and an inhibitor assay, we determined that these two genetic elements are part of a protein complex containing the scaffold protein CUL4. These findings thus suggest a photoprotective role for the putative E3 ubiquitin-ligase CUL4-DDB1^{DET1} in unicellular photosynthetic organisms that may mediate blue light signals to the photoprotective gene expressions. Further attempts to isolate mutants affecting the expression of *LHCSR1* and *LHCSR3*[6] and their characterizations will also be discussed.

References

1. Tokutsu, R. and Minagawa, J. (2013) *Proc Natl Acad Sci U S A* 110: 10016-10021.
2. Kim, E., Akimoto, S, Tokutsu, R, Yokono, M., Minagawa, J. (2017) *J Biol Chem* 292: 18951-18960.
3. Kosuge, K., Tokutsu, R., Kim, E., Akimoto, S., Yokono, M., Ueno, Y., Minagawa, J. (2018) *Proc Natl Acad Sci U S A* 115: 3722-3727.
4. Petroutsos, D., Tokutsu, R., Maruyama, S., Flori, S., Greiner, A., Magneschi, L., Cusant, L., Kottke, T., Mittag, M., Hegemann, P., Finazzi, G., Minagawa, J. (2016) *Nature* 537: 563-566.
5. Aihara, Y., Kamada-Fujimura, K., Yamasaki, T., Minagawa, J. (2019) *Nat Plants* 5: 34-40.
6. Tokutsu, R., Fujimura-Kamada, K., Matsuo, T., Yamasaki, T., Minagawa, J. (2019) *Sci Rep* 9: 2820.

> IL162. Invited Lecture
Symposium PLANT-3 Photoprotection in Photosynthetic Organisms (Diana Kirilovsky)

DYNAMIC FEEDBACK OF THE PHOTOSYSTEM II REACTION CENTRE ON PHOTOPROTECTION IN PLANTS

Authors: Herbert van Amerongen[1], Jevgenij Chmeliov[2], Leonas Valkunas[2], Gediminas Trinkunas[2], Arjen Bader[1], Rob Koehorst[1], Emilie Wientjes[1], Shazia Farooq[1]
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Photosystem II of higher plants is protected against light damage by thermal dissipation of excess excitation energy, a process that can be monitored through non-photochemical quenching of chlorophyll fluorescence. When the light intensity is lowered, non-photochemical quenching largely disappears on a time scale ranging from tens of seconds to many minutes. With the use of picosecond fluorescence spectroscopy, we demonstrate that one of the underlying mechanisms is only functional when the reaction centre of photosystem II is closed, that is when electron transfer is blocked and the risk of photodamage is high. This is accompanied by the appearance of a long-wavelength fluorescence band. As soon as the reaction centre reopens, this quenching, together with the long-wavelength fluorescence, disappears instantaneously. This allows plants to maintain a high level of photosynthetic efficiency even in dangerous high-light conditions.

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> IL165. Invited Lecture
Symposium PLANT-3 Photoprotection in Photosynthetic Organisms (Diana Kirilovsky)

REGULATION OF PHOTOSYNTHETIC LIGHT REACTIONS – AN EVOLUTIONARY PERSPECTIVE

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Linear electron transfer (LET) chain in the thylakoid membrane of oxygenic photosynthetic organisms is rather similar from cyanobacteria to higher plants. On the contrary, the light harvesting systems and a large number of various mechanisms regulating the distribution of excitation energy as well as the diversion of electrons from LET to different cyclic electron transfer routes or to molecular oxygen show distinct changes in the course of evolution. Development of chlorophyll-b-containing light-harvesting systems led to the segregation of the thylakoid membrane into PSII-rich appressed grana thylakoids and PSI-rich non-appressed stroma thylakoids. Light-induced dynamics in the lateral heterogeneity of the thylakoid membrane, together with activation of a number of different regulatory mechanisms, allow fluent photosynthetic electron transfer, equal light-harvesting capacity of both photosystems as well as efficient photoprotection in response to short-term changes in environmental cues. Photoinhibition of PSII and PSI plays a marked role in this regulatory network, and the production of specific reactive oxygen species in both photosystems have a distinct role in chloroplast retrograde signaling for long-term acclimation to environmental changes. An evolutionary overview of some specific photosynthesis regulation mechanisms will be discussed in the meeting.

> IL161. Invited Lecture

Symposium PLANT-3 Photoprotection in Photosynthetic Organisms (Diana Kirilovsky)

STRUCTURAL ADAPTATIONS OF PHOTOSYNTHETIC COMPLEX I ENABLE FERREDOXIN-DEPENDENT ELECTRON TRANSFER

Authors: Jan M Schuller[1], James M. Birrell[2], Hideaki Tanaka[3, 4], Tsuyoshi Konuma[5], Hannes Wulfhorst[6, 7], Nicholas Cox[2,8], Sandra K. Schuller[9], Jacqueline Thiemann[6], Wolfgang Lubitz[2], Pierre Sétif[10], Takahisa Ikegami[5], Benjamin D. Engel[11], Genji Kurisu[3, 4], Marc M. Nowaczyk[6]
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Photosynthetic complex I enables cyclic electron flow around photosystem I, a regulatory mechanism for photosynthetic energy conversion. We report a 3.3-Å resolution cryo-EM structure of photosynthetic complex I from the cyanobacterium *Thermosynechococcus elongatus*. The model reveals structural adaptations that facilitate binding and electron transfer from the photosynthetic electron carrier ferredoxin. By mimicking cyclic electron flow with isolated components in vitro, we demonstrate that ferredoxin directly mediates electron transfer between photosystem I and complex I, instead of using intermediates such as NADPH. A large rate constant for association of ferredoxin to complex I indicates efficient recognition, with the protein subunit NdhS being the key component in this process.

Reference

Schuller JM, Birrell JA, Tanaka H, Konuma T, Wulfhorst H, Cox N, Schuller SK, Thiemann J, Lubitz W, Sétif P, Ikegami T, Engel BD, Kurisu G, Nowaczyk MM (2018) **Science** 363:257-260

> IL163. Invited Lecture

Symposium PLANT-3 Photoprotection in Photosynthetic Organisms (Diana Kirilovsky)

CYANOBACTERIAL STATE TRANSITIONS

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Photosynthetic organisms, which should cope with changes in the quality and quantity of incoming light, need to sense and respond to these fluctuating environmental conditions, in order to perform efficient photosynthesis and to avoid the formation of dangerous reactive oxygen species. Cyanobacteria, like plants and algae, have developed a mechanism, named state transitions, that balances photosystem activities. State transitions are triggered by changes in the redox state of the membrane-soluble plastoquinone (PQ) pool. In plants and green algae, the reduction of the PQ pool induces the activation of a specific kinase via the cytochrome b6f complex that phosphorylates the membrane light harvesting complex II (LHCII). The phosphorylated LHCII detaches from PSII and attaches to PSI during transition from State I to State II. Oxidation of the PQ pool deactivates the kinase and a phosphatase dephosphorylates the LHCII that migrates again to the PSII. The migration of LHCII allows a readjustment in the distribution of excitation energy arriving at PSI and PSII. In cyanobacteria, this process, which involves fluorescence changes occurring upon illumination of dark-adapted cells or under illumination with light absorbed more specifically by PSII or PSI, remains an open question despite many studies resulting in several hypotheses and models. In this work, we characterize the role of the cytochrome b_6f and phosphorylation reactions in cyanobacterial state transitions using *Synechococcus elongatus* PCC 7942 and *Synechocystis* PCC 6803 as model organisms. First, a large Photosystem II fluorescence quenching was observed in State II which seems not to be related to energy transfer from Photosystem II to Photosystem I (spillover). This membrane-associated process was inhibited by betaine, sucrose and high concentrations of phosphate. Then, using different chemicals affecting the PQ pool redox state and the activity of the cytochrome b_6f, we demonstrated that this complex is not involved in *S. elongatus* and *Synechocystis* PCC6803 state transitions. Finally, by constructing and characterizing 21 protein kinase and phosphatase mutants and using chemical inhibitors, it was clearly shown that phosphorylation reactions are not essential in cyanobacterial state transitions. Thus, signal transduction is completely different in cyanobacterial and plant (green alga) state transitions.

> OC082. Oral Communication
Symposium PLANT-3 Photoprotection in Photosynthetic Organisms (Diana Kirilovsky)

NON-PHOTOCHEMICAL QUENCHING MECHANISMS IN VARIOUS PHOTOSYNTHETIC PROTEINS

Authors: Tomas Polivka[Unive], Hristina Staleva-Musto[Unive], Milan Durchan[Unive], Josef Komenda[Insti], Roman Sobotka[Insti], Francesco Saccon[Queen], Alexander Ruban[Queen]
Presenting Author: Tomas Polivka
1) University of South Bohemia

Non-photochemical quenching (NPQ) is an important process protecting the photosynthetic apparatus as it quenches excited states of chlorophyll molecules under excess light conditions. Mechanism of NPQ has been a subject of hot discussions since its discovery. It is well known that carotenoids play the key role in NPQ and two mechanisms directly involving carotenoids, energy transfer from excited chlorophyll to a carotenoid nearby or electron transfer generating carotenoid cation and chlorophyll anion which then recombines to form ground states of both molecules, have been suggested as two hot candidates for NPQ mechanisms. Here we compare NPQ mechanisms in the plants antenna protein LHCII, and in small proteins from either plants (LiL3) or cyanobacteria (Hlip) that play important photoprotective role during the assembly of the photosynthetic apparatus. We show that Hlips are locked in a quenched state that is achieved by specific interaction between protein and carotenoid (b-carotene in our case of Hlip from *Synechocystis*). This interaction causes significant decrease of energy of carotenoid excited states, allowing for carotenoids to take the energy from the excited chlorophyll. The efficient quenching in Hlips is achieved by fast (2 ps) energy transfer from chlorophyll to carotenoid. In plants, similar role have the light-harvesting like (LiL) proteins. We focused on LIL3 protein from *Arabidopsis thaliana* that binds chlorophyll and zeaxanthin which has, in comparison with LHCII, significantly red-shifted absorption spectrum with the reddest absorption band at 525 nm. Our study using ultrafast transient absorption spectroscopy shows that zeaxanthin in LiL3 behaves in a similar way as b-carotene in Hlips: after excitation of chlorophyll the energy is within a few picoseconds transferred to zeaxanthin. Finally, we compare these data with LHCII, in which we also see the carotenoid signals after excitation of chlorophyll, but the spectral and dynamical properties of the carotenoid signals differs from those observed in Hlips and LiL3. Our data suggest that a specific carotenoid conformation, induced by the binding site in each protein, is the key factor determining the ability to quench chlorophyll excited states. We show that in Hlips and LiL3, which are likely designed to protect under any light conditions, the protein is locked in the quenching conformation, while LHCII has the ability to switch between quenched and non-quenched conformations.

> OC083. Oral Communication
Symposium PLANT-3 Photoprotection in Photosynthetic Organisms (Diana Kirilovsky)

MOLECULAR ANATOMY OF PLANT PHOTOPROTECTIVE SWITCHES: THE SENSITIVITY OF PsbS TO THE ENVIRONMENT, RESIDUE BY RESIDUE

Authors: Nicoletta Liguori[1], Sara R.R. Campos[2], António M. Baptista[2], Roberta Croce[1]
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Under strong sunlight, plants avoid photooxidation by dissipating excess absorbed energy as heat. This quenching process occurs in the photosynthetic membranes (thylakoids) and is triggered by a membrane protein called PsbS. However its mechanism of action is still unknown. The activation and deactivation of the quenching process are regulated by the pH of the thylakoid lumen, which becomes more acidic in high light, when photosynthesis is saturated. Several glutamic residues of PsbS were shown to be important for this activation, suggesting that they can act as pH sensors. However, the pKa of glutamate is several pH units below the values reached in the lumen in physiological conditions.

How can thus PsbS sense the pH of the lumen? And what is its response to the changes in pH?

By applying a non-standard molecular dynamics (MD) method that treats pH explicitly, we show that the lumen-exposed residues have strongly shifted pKa values and that such shifts are crucial for the pH-sensitivity of PsbS in physiological conditions. We also demonstrate that protonation drives a systematic unfolding of a region involved in protein-protein interactions, suggesting that PsbS responds to the acidification of the thylakoid lumen via a functional conformational switch.

> P094. Poster

Symposium PLANT-3 Photoprotection in Photosynthetic Organisms (Diana Kirilovsky)

NADK3, ONE OF NAD KINASES HAS A PRINCIPAL ROLE IN PHOTORESPIRATION IN ARABIDOPSIS

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Nicotinamide adenine dinucleotides (NAD^+ and $NADP^+$) are electron mediators involved in various metabolic pathways. One of enzymes which regulates phosphorylation ratio (NADP(H)/NAD(H)) is a NAD kinase (NADK). NADKs have been found in all organisms examined to date, suggesting a fundamental role in cells. In the photosynthesis, NADPH is produced by the ferredoxin-NADP reductase in the last step of the photosynthetic linear electron transport chain in the thylakoid membrane of chloroplast. $NADP^+$, the electron acceptor, is supplied by the NADK through the phosphorylation of NAD^+. Arabidopsis has three NADKs (NADK1, At3g21070; NADK2, At1g21640, and NADK3, At1g78590) in its genome. NADK1 is in cytosol, NADK2 is localized in chloroplast, and NADK3 is reported to be localized in peroxisome. To evaluate the specific role of each NADK in metabolic pathways, we conducted metabolome analysis of NADK mutants (*nadk1, nadk2,* and *nadk3*) in Arabidopsis, and found that glycine and serine, which are intermediates of photorespiration, were specifically accumulated in the *nadk3* mutant. The *nadk3* mutant appeared growth retardation under normal growth condition (8h light/12h dark, 120 µmol/m^2/s light, ambient air). NAD(H) contents in the *nadk3* mutant were increased, but redox status ((NAD(P)H/NAD(P)) was not changed in the *nadk3* compared to the wild-type plant. To evaluate the photorespiration, post-illumination CO_2 burst (PIB) was examined under low CO_2 (0.012%) with high light (1000 µmol/m^2/s) conditions. The *nadk3* plant grown under normal growth condition showed the decreased PIB compared to the control plants, indicating disturbed photorespiration in the *nadk3.* Furthermore, both glycine and serine contents, which were increased in the *nadk3* grown under the normal condition, were decreased when the *nadk3* plant was treated with high CO_2 (0.15%) for 4 h. These data indicate that the peroxisome localizing NADK3 has the fundamental function in photorespiration through the NAD(P)(H) metabolism of peroxisome.

> IL166. Invited Lecture
Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

HOW DO PLANTS RESPOND TO UV-B IN NATURAL GROWTH ENVIRONMENTS?

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UV-B wavelengths initiate a range of regulatory responses in plants that modify morphology, metabolism and physiology, and include changes in biochemical composition that promote UV-protection and defence against pests and pathogens. UV RESISTANCE LOCUS8 (UVR8) is the only photoreceptor known to mediate photomorphogenic responses to UV-B. UVR8 signaling leads to the regulation of transcription of numerous genes that underpin responses. Most of our knowledge of UVR8 function has come from experiments with purified protein and exposure of non-acclimated plants to UV-B. However, it is important to understand how UVR8 functions in UV-B-acclimated plants under realistic growth conditions.

UVR8 exists as a homodimer in the absence of UV-B and UV-B photoreception causes rapid dissociation of the dimer into monomers to initiate signaling and hence gene expression through interaction with the CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) protein and a number of transcription factors REPRESSOR OF UV-B PHOTOMORPHOGENESIS (RUP) proteins promote reversion of monomers to the dimer. Under photoperiodic illumination with white light supplemented with UV-B a dimer/monomer photoequilibrium is established, where approximately 70% of UVR8 is in the dimeric form. Factors that influence the photoequilibrium will modulate UVR8 function in natural growth environments.

UVR8 has maximal absorption at approximately 280 nm but sunlight does not contain wavelengths below ~290 nm. The action spectrum for UVR8-mediated responses peaks at approximately 300 nm. It is therefore important to consider how UVR8 functions under natural spectral qualities.

In addition, UV-B regulates the expression of many genes independently of UVR8. Transcriptomic analysis shows that very low fluence rates of UV-B can initiate gene expression responses in *uvr8* mutant plants. The signaling pathways involved in these responses are unknown.

> IL167. Invited Lecture
Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

ROLE OF UVR8 PHOTORECEPTOR IN SUNLIGHT

Authors: Neha Rai[1], Andrew O'Hara[2], Daniel Farkas[2], Åke Strid[2], Pedro J. Aphalo[1], Luis O. Morales[1,2]
Presenting Author: Neha Rai
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In plants, light perceived through photoreceptors regulates growth, development, and acclimation to the environment. UV RESISTANCE LOCUS 8 (UVR8) and CRYPTOCHROMES 1 and 2 (CRYs) are known to play major roles in the perception of UV-B (280–315 nm) and UV-A/blue radiation (315–500 nm), respectively. However, how these photoreceptors regulate gene expression response in sunlight is poorly understood. To address this, we performed an experiment with *Arabidopsis thaliana* wild-type and UVR8 and CRYs photoreceptor mutants. Plants were exposed to sunlight for 6 h or 12 h under five types of filters with cut-offs at different wavelengths in UV and blue light regions. The regulation of gene expression by UV-B and UV-A wavelengths shorter than 350 nm (UV-A_{sw}) required UVR8 whereas regulation by blue and UV-A wavelengths longer than 350 nm (UV-A_{lw}) required CRYs. These results agree with our estimates of sunlight photons absorbed by the photoreceptors. UVR8 monomerized at wavelengths between 300 nm and 335 nm, which agrees with the role of UVR8 in UV-A. In addition, the number of genes differentially expressed in response to UV-B and UV-A_{sw} in the absence of CRYs was three times that in the wild type. Thus, we provide strong evidence that UV-A_{sw} perception in plants is mediated by UVR8 and that an asymmetric antagonistic interaction exists between CRYs and UVR8 in sunlight.

Acknowledgements
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> IL168. Invited Lecture
Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

SIGNAL TRANSDUCTION MEDIATED BY THE PLANT UV-B PHOTORECEPTOR UVR8

Authors: Tong Liang[Insti], Yu Yang[Insti], Hongtao Liu[Insti]
Presenting Author: Yawen Liu
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Ultraviolet-B (UV-B) light is an intrinsic part of sunlight that has significant effects on plant development and acclimation responses. UVR8 (UV Resistance Locus 8) is the long sought-after UV-B photoreceptor that mediates UV-Blightperception and signal transduction.UV-Birradiation induces the monomerization and nuclear accumulation of UVR8 inplant cells to activate the UV-B signaling pathway.The photoactivated UVR8 could transduce UV-B signal via multiple mechanisms to regulate transcription and plant growth. Here, we summarize current understanding of UVR8mediated UV-B signal transduction pathways, including UVR8–COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC 1) and UVR8–WRKY36 (WRKY DNA-BINDING PROTEIN 36), UVR8–BES1 (BRI1-EMS-SUPPRESSOR1) and BIM1 (BES1-INTERACTING MYC-LIKE 1)

> IL169. Invited Lecture
Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

E2Fc AND E2Fb TRANSCRIPTION FACTORS INDEPENDENTLY REGULATE PLANT GROWTH UNDER UV-B CONDITIONS IN ARABIDOPSIS THALIANA.

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UV-B radiation inhibits plant growth, and this inhibition is, to a certain extent, regulated by the activity of the E2Fe transcription factor. E2Fe is a target of regulation by two transcription factors from the same family, E2Fb and E2Fc. While E2Fc acts as a repressor, E2Fb is a transcriptional activator of E2Fe. Therefore, we investigated if the modulation of UV-B responses by E2Fe is through its regulation by E2Fb and/or E2Fc. We found that, at UV-B intensities that induce DNA damage, inhibition of cell proliferation is regulated by both E2Fc and E2Fb. E2Fc controls leaf size under UV-B regulating DNA damage responses, as E2Fc deficient plants show decreased programmed cell death in the roots after exposure and altered SOG1 and ATR expression. Moreover, E2Fc has an epistatic role over the miR396 pathway under UV-B, which also regulates leaf growth under these conditions. On the other hand, although E2Fb also controls cell proliferation under UV-B conditions; it does not regulate programmed cell death in the roots after exposure. Interestingly, E2Fb deficient leaf cells have increased DNA ploidy levels after UV-B exposure, similarly as E2Fe deficient cells. Together, our results demonstrate that E2Fc is required for miR396 activity on cell proliferation under UV-B, and that its role is independent of E2Fe, probably modulating DNA damage responses through the regulation of SOG1 and ATR levels. On the contrary, the regulation of DNA ploidy in leaf cells under UV-B previously described in E2Fe deficient plants could be regulated by E2Fb activity.

> IL170. Invited Lecture
Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

SPECTRAL CUES PROMPTING DIURNAL CHANGES IN LEAF PIGMENTS

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Introduction
Plants possess some intrinsic diurnal cycles whilst others are controlled by spectral cues. Recently, daily changes in epidermal transmittance of UV radiation have been recorded and are thought to be a mechanism by which plants can moderate the UV radiation reaching the leaf mesophyll at solar noon when UV-B radiation peaks (Barnes et al., 2017). This diurnal pattern is linked with the accumulation of flavonoid compounds which is a well-established UV response over time scales of days to months (Barnes et al., 2016). However, the cues underlying this daily pattern of epidermal transmittance are yet to be experimentally determined. This diurnal response in flavonoids accompanies those of chlorophylls, carotenoids, and xanthophyll-cycle pigments which have also been found to follow distinctive daily cycles (García-Plazaola et al., 2017, FernándezMarín et al., 2018).

Methods
Here, we show the first results from a spectral attenuation experiment at an EU Long Term Ecological Research station (eLTER) at the Station Alpine du Lautaret. Alpine species were compared under plastic filters that differentially attenuated different portions of the solar spectrum: either UV-B; UV-A and –B; blue and UV; or the entire spectrum. The diurnal patterns of leaf pigments were traced throughout the day using a non-destructive optical leaf-clip sensor (Dualex Scientific +), and verified against concentrations of pigments calculated from biochemical analysis of leaf extracts. Following these time-series changes allowed the spectral cues responsible for the diurnal patterns in epidermal transmittance to be identified.

Results and Discussion
Species-specific differences in the extent of epidermal screening of UV radiation suggest different strategies persist for plants of different origin to deal with high solar irradiances at high elevations. Concomitant changes in a suite of leaf pigments active in photosynthesis, photoprotection, and antioxidant systems, also imply that plants have a coordinated response functioning through a combination of photoreceptors that work in different regions of the solar spectrum. This understanding allows us to start to differentiate the relative importance of blue light and UV radiation in maintaining an appropriate and dynamic responses suitable for changing the light environment of a plant.

Conclusions
Changes in optical properties of leaves affecting UV-transmittance through the day reflect effective fine-tuning to small changes in temperature and irradiance that help to maintain efficient photosynthetic function. The maintenance of high photosynthetic efficacy in alpine species is an indication of the effectiveness of these responses in coping with high irradiances which are enriched in UV-B radiation compared with lower elevations.

Acknowledgements
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> IL171. Invited Lecture
Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

USING SUPPLEMENTARY UV RADIATION TO IMPROVE PRODUCTION OF GREENHOUSE CROPS: THE EXAMPLE OF CUCUMBER (CUCUMIS SATIVUS)

Authors: Minjie Qian[1], Eva Rosenqvist[2], Irina Kalbina[1], Ann-Marie Flygare[1], Marcel A.K. Jansen[3], Åke Strid[1]
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1) Örebro University 2) University of Copenhagen 3) University College Cork

Introduction
In northern Europe a large number of vegetable and herbal crops are produced in greenhouses. The cladding material strongly absorbs the UV part of the incoming solar radiation. This means that important morphogenic UV radiation is missing, unless expensive UV transparent material is used. For smaller firms it may be more affordable to replace the sun's UV radiation with supplementary UV inside the greenhouse. In this cucumber study we used supplementary UV-A or UV-B radiation to increase quality of the produce and increase the economic efficiency of the production.

Methods
UV-A- and UV-B-enriched light was used to illuminate cucumber seedlings in a greenhouse for 4 h daily. Morphological parameters were measured with six technical and three biological repeats in each single experiment. In addition, these experiments were repeated three times. After 14 days of UV exposure, plants were transferred to a commercial cucumber grower (using 800 ppm CO_2) to study the effect of the supplementary UV exposures on the final yield of harvest.

Results and Discussion
Generally, both UV-A- and UV-B-enriched light led to a number of morphological changes including reduced plant height and smaller leaves. The effect of UV-B was greater than that of UV-A. Whereas the changes after UV-A exposure resulted in more robust plants (thicker leaves, stiffer internodes) with an increased root-to-shoot ratio, the opposite was seen for UV-B. In the commercial setting no difference was seen in fruit yield between control plants and plants treated with supplementary UV-enriched light.

Conclusions
UV supplementation can be used to produce more compact seedling plants that use less space for growing and for transport and thereby would also be more efficient from a producer's energy consumption perspective. Since the final fruit yield was not negatively affected, this could lead to a better economy for the seedling grower's business. Also, since UV-A-exposed plants became more robust, such a treatment would result in less transport damage.

Acknowledgements
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Conflicts of Interest
The research was carried out in collaboration with cucumber producers. The research question had a foundation in their core activities. However, the companies had no influence on the formulation of the research question, the methods chosen, or the analysis and interpretation of the data. Any economical contributions from the producers were 'in kind'.

> OC084. Oral Communication
Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

CROSS-REGULATION BETWEEN UV-B AND VISIBLE LIGHT SIGNALLING IN ARABIDOPSIS

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Perception of light and its integration with diverse environmental signals and developmental programs involves complex molecular mechanisms. UV-B is a potentially harmful part of the solar spectrum that is perceived by the UV-B photoreceptor UVR8 (UV RESISTANCE LOCUS8), necessary for UV-B acclimation and stress tolerance. UVR8 is a homodimer in its ground state that monomerizes upon UV-B perception. Redimerization of UVR8 is facilitated by RUP1 and RUP2 (REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 & 2) in a negative feedback loop. We identified RUP1 and RUP2 as molecular actors that constitute a direct cross-talk node between visible and UV-B light signalling and how this may contribute to an overall balanced photoprotection. Using a combination of molecular, biochemical, genetic and physiological approaches and methods, we characterized the impact of visible light on UV-B signalling and responses. Conversely, we tested whether UV-B modulates visible light signalling pathways. We will present data for the molecular integration of visible and UV-B light signalling in plants, and discuss the implications for plant survival in a multichromatic environment.

> OC085. Oral Communication
Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

IMPROVING THE STRESS TOLERANCE OF PEPPER SEEDLINGS VIA MANIPULATING SECONDARY METABOLITES WITH UV IRRADIATION

Authors: Kristóf Csepregi[Unive], Gyula Czégény[Unive], Arnold Rácz[Unive], Éva Hideg[Unive]
Presenting Author: Kristóf Csepregi
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Transplantation of seedlings from indoor nurseries to outdoor conditions is a common agricultural practice for several species. This sudden change in light, temperature and other abiotic factors may lead to oxidative stress as a result of inadequate antioxidant protection. Secondary metabolites may promote stress tolerance as antioxidants and epidermal filters, and their biosynthesis can be stimulated by controlled UV-B (280-315 nm) irradiation (Schreiner et al, 2014). Higher phenolic contents result in increased non-enzymatic antioxidant capacities (Csepregi et al, 2016) and UV-B exposure of lettuce seedling has been shown to improve their photoprotection and long term development (Wargent et al, 2011). The present work is aimed at the biofortification of young pepper plants by UV-B. Bell pepper (Capsicum annum var. grossum) seedlings were grown in growth chambers (25°C/20°C, 16h/8h day/night) and the effect of UV-B pretreatment on responses to a consecutive cold stress was studied measuring leaf photosynthesis, pigment content and antioxidant capacities. One month old pepper seedlings were exposed to 6.9 kJ $m^{-2}d^{-1}$ biologically effective UV-B (Q-Panel UVB-313EL tubes) for 5 days before a 5-day long cold treatment (15°C/10°C). Leaf photochemistry was characterized by chlorophyll-fluorescence-derived yield parameters, adaxial and abaxial pigment contents were estimated by using Dualex Scientific+. Total antioxidant capacities were assayed spectrophotometrically. For modelling natural events during transplantation, we recorded chlorophyll fluorescence parameters under saturating light conditions up to 800 µmol $m^{-2}s^{-1}$ PAR. UV pretreatment did not affect photochemical electron transport rate (ETR) but increased leaf flavonoid content. UV-B improved leaf antioxidant properties and resulted in a more successful acclimation of pepper seedlings to subsequent low temperature as demonstrated by more effective ETR in these leaves than the ones exposed to stress without the pre-treatment. Supported by the National Research, Development and Innovation Office (NN128806).

References

Csepregi, K.; Neugart, S.; Schreiner, M.; Hideg, É. Comparative evaluation of total antioxidant capacities of plant polyphenols. *Molecules*. 2016, 21, 208

Schreiner, M.; Mewis, I.; Huyskens-Keil, S.; Jansen, M.A.K.; Zrenner, R.; Winkler, J.B.; O'Brien, N.; Krumbein, A. UV-B induced secondary plant metabolites-potential benefits for plant and human health. *Plant Sci*. 2012, 31, 229-240

Wargent, J.J.; Elfadly, E.M.; Moore, J.P.; Paul, N.D. Increased exposure to UV-B radiation during early development leads to enhanced photoprotection and improved long-term performance in *Lactuca sativa*. *Plant Cell Environ*. 2011, 34, 1401-1413

> OC086. Oral Communication
Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

LET THE SUNSHINE IN! POST-HARVEST UV-B RADIATION IS ABLE TO AFFECT THE SECONDARY METABOLISM IN FLESH OF PEACH FRUIT

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The greatly appreciated effect of UV-B radiation in promoting phenolics accumulation, depending on the UV-B dose and the phenolic class considered, has been already elucidated in many fruit and vegetables [1]. Previous studies reported that 10 min and 60 min of UV-B irradiation were effective in stimulating a strong phenolic accumulation in peach, especially dihydroflavonols, anthocyanins and flavones, which are among the strongest antioxidant phytochemicals within the phenolics [2,3]. However, almost the entire relevant literature has considered the UV-B-driven phenolics changes only in the fruit skin, since it represents the outermost tissue and therefore directly exposed to the UV-B radiation. It is also important to point out that most people use to peel the fruit due to the possible presence of harmful chemicals, e.g. pesticides and fungicides, thus they would not benefit from the phenolics enrichment occurring in the skin. In the light of above, and considering the scarcity of current literature about an "-omics" approach to investigate the UV-B effects on secondary metabolism, this work aimed to figure out whether the UV-B exposure might influence the secondary metabolism within the peach flesh, focusing particularly on phenolic compounds. Based on these considerations, melting flesh yellow peaches (Prunus persica L., cv. Fairtime) were exposed to UV-B radiation (2.31 W m^{-2}) for 10 and 60 min, and the flesh was sampled at two different recovering times, 24 and 36 h. Through UHPLC-ESI/QTOF-MS followed by a fold-change analysis, we were able to find which metabolites were mostly affected by UV-B radiation in the flesh. Phenolics compounds were highly affected by UV-B radiation, showing an initial slight decrease after 24 h from the irradiation, and later an accumulation after 36 h. Since this behaviour reflects what has been already observed in the skin, a possible transduction mechanism of the UV-B signal from the skin to the flesh below is likely to occur. Indeed, nowadays, no studies have measured the UV-B transmittance within the peach skin, although it has been previously found that UV transmittance across tomato peel is only about 0.5%. Besides phenolics, terpenoids were also highly affected by UV-B radiation, showing a great increase of most terpenoid subclasses, especially after 36 from the treatments. In detail, carotenoids showed the highest increase among terpenoids after both 24 and 36 h recovery timepoints. Individual UV-B-responsive metabolites will be further discussed. These findings pave the way for a possible application of UV-B irradiation to increase the nutraceutical value of plant products in the view of a sustainable food chain.

References

1. M. Schreiner et al., *Opt. Photonik*, 2014, **9**, 34–37.
2. M. Santin et al., *Plant Physiol. Biochem.*, 2019, **135**, 511–519.
3. M. Santin et al., *Postharvest Biol. Technol.*, 2018, **139**, 127–134.

> P095. Poster

Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

DIFFERENT IRRADIANCES OF UV AND PAR IN THE SAME RATIOS ALTER THE FLAVONOID PROFILES OF ARABIDOPSIS THALIANA WILD-TYPES AND UV-SIGNALLING PATHWAY MUTANTS

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The UVR8 photoreceptor in *Arabidopsis thaliana* is specific to ultraviolet-B (UV-B; 280-315 nm) radiation and its activation leads to a number of UV-B acclimation responses, including the accumulation of flavonoids. UVR8 is involved in a signalling cascade that includes COP1, HYH5 and HYH such that the lack of any one of these components leads to a reduction in a plant's ability to accumulate flavonoids in response to UV; high drop-outs are evident in *cop1* mutants and very low concentrations of flavonoids occur in *hy5-kss50hyh* double mutants. The predominant phenolics in *Arabidopsis thaliana* are sinapic acid derivatives as well as non-aclyated quercetin and kaempferol di- and triglycosides containing glucose and rhamnose as glycosylated sugar moieties. How this flavonoid profile in *Arabidopsis thaliana* is influenced by UV, how quickly these changes occur when UV conditions change, and what components of the UV-B signalling pathway are involved in rapid acclimation responses to UV is poorly understood. In the present study, we explored these questions by characterizing the flavonoid profiles of *Arabidopsis thaliana* signalling mutants and wildtypes grown under different UV levels of constant UV-B+PAR ratios and then transferring a subset of plants to alternate UV conditions. Results indicate that flavonoid accumulation in *Arabidopsis thaliana* is triggered by UV and this response is amplified by higher levels of UV but not to the same degree by all compounds. The catechol structure in quercetin seems to be less important than the glycosylation pattern, e.g. having 2 rhamnose moieties in determining responsivity. At low UV+PAR intensities the introduction of UV leads to an initial increase of flavonoids in the wild-types that was detected after 3 days. It took 7 days for these changes to be detected in plants grown under high UV+PAR intensities suggesting a priming of PAR. Thus, the flavonoid profile in *Arabidopsis thaliana* is altered over time following exposure to UV and PAR, but the functional significance of these changes is unclear at present.

> P096. Poster

Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

INTERACTIONS OF UV-B WITH OTHER FACTORS AFFECTING LEAF ANTIOXIDANTS

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Plants are exposed to a multi-factor environment outdoors in which every variable is a potential stressor. Increased levels of antioxidants are one aspect of well-characterized acclimatory responses and the success of acclimation to multi-factor stress is affected by interactions between single-factor responsive pathways. As part of a series investigating the potential regulatory role of UV-B light, interactions between antioxidant responses to UV-B (Czégény et al. 2016) and to other factors were studied in *Nicotiana* plants. In the first experiment UV-B was combined with the soil application of β-aminobutyric acid (BABA), which is a potential novel plant hormone, capable of inducing resistance against a variety of abiotic stresses (Cohen et al. 2016). In the second experiment, UV-B was combined with drought, which is also known to evoke antioxidant responses (Chaves et al. 2003).

Plants for both experiments were cultivated in growth chambers under 175 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR), 25/20°C (16h/8h day/night), 70% RH. Treatments started 4 weeks after emergence. Drought was achieved by limited watering and lowering RH to 50%. This resulted in a 40% loss of soil water content within 2 days, which was maintained for 8 more days. Drought was applied either as single factor or together with supplementary UV-B (6.9 kJ m^{-2} d^{-1} b.e.d.). In the second experiment, 300 ppm BABA solution was applied as soil drench and this pre-treatment was followed by exposure to UV-B for 8 days.

Drought as single factor resulted in elevated flavonoid content, higher total antioxidant capacities (TAC) and increased non-enzymatic H_2O_2 neutralization. Supplementary UV-B enhanced these responses in the two-factor experiment, with the exception of non-enzymatic H_2O_2 scavenging, which was non-responsive to UV-B as single factor either (Mátai et al. 2019a). In the second experiment, BABA as single factor increased TAC without affecting the flavonoid content, increased non-enzymatic H_2O_2 neutralization, but decreased $^{\cdot}OH$ scavenging. BABA pre-treatment had a lasting effect and modified leaf responses to consecutive UV-B. In this two-factor experiment TAC responses to BABA and UV-B were additive, but the positive effect of UV-B on $^{\cdot}OH$ neutralization overrode the opposite effect of BABA. (Mátai et al. 2019b). These results illustrate that UV-B light is capable of modulating leaf antioxidant responses to other factors, and that BABA may be a useful diagnostic tool to dissect the complexity of the UV-B responses.

References

Chaves MM, Maroco JP, Pereira JS (2003) Funct Plant Biol 30: 239–264.
Cohen Y, Vaknin M, Mauch-Mani B (2016) Phytoparasitica, 44: 513–538.
Czégény Gy, Mátai A, Hideg É (2016) Plant Sci 248: 57–63.
Mátai A, Nagy D, Hideg É (2019a) Plant Physiol Biochem 134: 9–19.
Mátai A, Jakab G, Hideg É (2019b) Photochem Photobiol Sci 18: 359–366.

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> P097. Poster

Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

PHYSIOLOGICAL AND MOLECULAR FUNCTION OF PHOTORECEPTOR UVR8 IN THE LIVERWORT *MARCHANTIA POLYMORPHA*

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Introduction

UV RESISTANCE LOCUS 8 (UVR8) is a photoreceptor for UV-B discovered in *Arabidopsis thaliana*and other species. To understand the mechanisms of UV-B sensing and tolerance common to land plants, we have been investigating the physiological and molecular function of UVR8 in the liverwort, *Marchantia polymorpha*, which belongs to the earliest diverging group of embryophyte lineages. Here, using the Mp*UVR8*-disrupted mutant (Mp*uvr8*^{ko}), we characterized the physiological function of *M. polymorpha* UVR8 (MpUVR8) and UV-B tolerance induced by MpUVR8-depdendent and independent signaling pathways in *M. polymorpha*.

Methods

We produced the Mp*uvr8*^{ko}, in which a hygromycin cassette was inserted into the Mp*UVR8* locus by gene-targeting. For white light and UV-B condition, 45 µmol $m^{-2}s^{-1}$ white light and 45 µmol $m^{-2}s^{-1}$ white light supplemented with 1.3 Wm^{-2} UV-B light obtained from FL20SE UV-B fluorescent tubes were used, respectively.

Results and Discussion

To investigate the tissue specific expression pattern of Mp*UVR8*in thalli of *M. polymorpha*, ß-glucuronidase (GUS) gene was driven under the control of a Mp*UVR8* promoter region containing 2.5 kbp upstream of translational start site in the wild type, Tak-1. The GUS activity was higher in the thalli meristematic zones of the thalli during developmental stages. This result suggests an immediate and strong response to UV-B irradiation to counteract of the inhibition of DNA replication in these tissues. Mp*uvr8*^{ko} plants showed growth retardation in comparison with Tak-1 plants under the UV-B condition despite the fact that Mp*uvr8*^{ko} thalli grew similarly to Tak-1 thalli under the white light condition. Abundance of UV-B-absorbing compounds was less in Mp*uvr8*^{ko} plants than that in Tak-1 plants under the UV-B condition. The expression levels of *M. polymorpha ELONGATED HYPOCOTYL 5* (Mp*HY5*), *M. polymorpha CHALCONE SYNTHASE* (Mp*CHS*) and *M. polymorpha MYB14* (Mp*MYB14*) were elevated in response to UV-B irradiation in Tak-1, while they were significantly decreased in Mp*uvr8*^{ko}. These results suggest that MpUVR8 promotes the expression of these genes to induce the accumulation of UV-B-absorbing compounds under UV-B condition in *M. polymorpha*. On the other hand, the expression levels of Mp*HY5* and Mp*MYB14* were elevated in both Mp*uvr8*^{ko} and Tak-1 thalli after long exposure to UV-B light, suggesting the important roles of MpUVR8-depdendent and independent signaling pathways for UV-B tolerance. Moreover, subcellular localization of MpUVR8 was also investigated in transgenic plants expressing the gene encoding Citrine-fused MpUVR8. As in the case of *A. thaliana* UVR8, the UV−B dependent translocation of MpUVR8 from cytosol to nucleus was observed.

Conclusion

We have demonstrated strong conservation of the physiological and molecular function of UVR8 to regulate transcription of various genes related with UV-B tolerance in embryophytes.

> P098. Poster

Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

REGULATION OF UVR8 ACTION

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The photoreceptor UVR8 (UV RESISTANCE LOCUS 8) mediates photomorphogenic responses to UV-B in plants. UV-B photoreception initiates dissociation of UVR8 dimers to monomers that accumulate in the nucleus. Interaction of the UVR8 monomer with COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC1) leads to accumulation of the HY5 transcription factor, which is involved in many photomorphogenic UV-B responses. Among the genes expressed by the UVR8-COP1-HY5 signaling pathway are those encoding RUP1 (REPRESSOR OF UV-B PHOTOMORPHOGENESIS1) and RUP2, which promote re-dimerisation of UVR8 monomers and are therefore negative regulators of UVR8 mediated responses.

We have studied how UVR8 dimer/monomer status and activity is regulated through interaction with COP1 and RUP proteins. We have measured changes in interaction of UVR8 with COP1 and RUP proteins by co-immunoprecipitation, and in the abundance of these proteins when plants are transferred to UV-B. Since the RUP proteins are important regulators of UVR8 action, we examined the expression of RUP1 and RUP2 by immunodetection with specific antibodies. The results add to present understanding of UVR8 action.

> P099. Poster

Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

CHLOROPLASTS EXHIBIT ACCUMULATION RESPONSE AFTER UVB IRRADIATION IN A PHOTOTROPIN – DEPENDENT MANNER

Authors: Pawel Hermanowicz[1], Agnieszka Katarzyna Banaś[2], Olga Sztatelman[3], Halina Gabrys[2], Justyna Łabuz[1]
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Introduction

Chloroplasts relocate in response to changing light conditions. In weak light, they accumulate at the cell walls perpendicular to the light direction. In high light, they move towards the walls parallel to the light direction, protecting the photosynthetic apparatus from damage. Chloroplast movements require the actin cytoskeleton and are triggered by phototropins (phot1 and phot2 in *Arabidopsis thaliana*), which use FMN as a chromophore. Phot2 mediates both responses, while phot1 can trigger only the accumulation response. Phototropins are typically described as blue/UVA photoreceptors. We examined the effects of UVB irradiation on chloroplast movements in *A. thaliana*.

Methods

Leaves were irradiated for 1 h with UV, supplied with UVB fluorescent tubes and additionally filtered to obtain the wavelength range of 280 - 325 nm. Chloroplast relocations were examined using a microscope and by measurements of changes in the leaf transmittance using a double beam photometer[1]. The effect of UVB on the structure of the actin cytoskeleton was examined in an *Arabidopsis* line expressing LifeAct-GFP[2]. The content of UV-absorbing compounds in the epidermis was assessed using Dualex.

Results and conclusions

Irradiation with 3.3 µmol m^{-2} s^{-1} (1.3 W m^{-2}) of UV induced substantial chloroplast accumulation in wild type leaves. A similar response was observed in the *uvr8* mutant. No directional chloroplast movement was observed in the *phot1phot2* mutant, suggesting that UVB-induced chloroplast accumulation depends on phototropin, but not on UVR8. Accumulation was stronger in the *phot2* mutant than in the wild type and barely detectable in the *phot1* mutant. The magnitudes of the responses to UVB and to blue light (455 nm) of the same intensity were comparable. No substantial difference in the epidermal UV transmittance of the analyzed mutants was observed. Strong UV of 20 µmol m^{-2} s^{-1} (7.8 W m^{-2}) induced accumulation only in the *phot2* mutant, but not in the wild type. However, wild type leaves pretreated with strong UV exhibited chloroplast movements upon subsequent illumination with blue light. In addition, strong UV induced chloroplast avoidance in the accumulation-defective *jac1* mutant. This indicates that the absence of chloroplast responses to strong UV in wild type leaves did not result from non-specific damage, but from the balance between competing signals to chloroplast accumulation and avoidance. Irradiation with UVB of 3.3 µmol m^{-2} s^{-1} did not disrupt the actin cytoskeleton in the LifeAct-GFP line, but strong UVB affected its structure. The expression of phot2, but not phot1, was induced by UV, in a UVR8 dependent manner, at the mRNA level.

Acknowledgements

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References

[1] Walczak T, Gabrys H. Photosynthetica 14: 65–72.
[2] Smertenko A, Deeks MJ, Hussey PJ 2010. J Cell Sci, 123(17), 3019-3028.

> P100. Poster

Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

ANALYSIS OF A PUTATIVE PHOTOLYASE ENCODED BY At4g25290

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Most organisms including plants use photolyases to cope with UV-induced pyrimidine dimers in their DNA. Photolyases are enzymes which use blue light/UVA energy to reverse dimerization of pyrimidines. They belong to one protein family with blue light photoreceptors - cryptochromes (crys). Besides well characterized photolyases, two genes, *AtPHR2* and *At4g25290,* encoding proteins with putative photolyase activity have been identified in the *Arabidopsis* genome. In addition to the N-terminal DNA photolyase related domain and a flavin adenine dinucleotide binding domain At4g25290 has also a C-terminal hydrolase domain with an unknown substrate specificity. Such an expanded C-terminus is typical for cryptochromes.

When expressed in *Escherichia coli* lacking their own photolyases, *At4g25290* enhanced bacteria survival after UV-treatment. This effect was only slightly stronger when photoreactivating light was applied after UV irradiation. *At4g25290* transcripts were found in *Arabidopsis* leaves, stems, flowers, siliques and roots, however their levels were lowest in the later organ. Illumination with visible light up-regulated *At4g25290* expression at the mRNA and protein levels. This effect was observed even when photosynthesis was blocked. *Arabidopsis* cry1 and cry2 redundantly up-regulated the amount of *At4g25290* under blue light. The photoreceptor acting under red light was not identified. An increase in *At4g25290* mRNA level after UV-B light treatment was partially dependent on the UV-B photoreceptor, UVR8. GFP-tagged At4g25290 localized to chloroplasts in transiently transformed *Nicotiana benthamiana* and in stable transgenic *Arabidopsis* lines. It co-localized with PEND (plastid envelope DNA-binding protein) suggesting the involvement of At4g25290 in maintenance of chloroplast DNA. This co-localization was independent of UV-B irradiation. Thus, it was not a consequence of binding to pyrimidine dimers by the photolyase domain of At4g25290. In line with those results, no differences in photorepair between wild type (WT) and *At4g25290* mutant plants were observed. To test whether At4g25290 may be involved in the repair of other DNA lesions, ciprofloxacin, an antibiotic introducing double strand breaks into chloroplast DNA was used. Surprisingly, the survival of ciprofloxacin-treated plants either overexpressing *At4g25290* or having T-DNA insertion in this gene was higher than in WT ones. However, the function of *At4g25290* remains still unclear.

Acknowledgments

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> P101. Poster

Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

LEAF ANTIOXIDANT RESPONSES TO EXOGENOUS AND TO PHOTOBIOLOGICALLY GENERATED ENDOGENOUS HYDROGEN PEROXIDE

Authors: Arnold Rácz[1], Gyula Czégény[1], Éva Hideg[1]
Presenting Author: Arnold Rácz
1) University of Pécs Faculty of Sciences

Acclimative plant responses to supplemental UV-B (280-315 nm) radiation were shown to increase peroxidase enzyme activities in leaves (Czégény et al. 2016, Rácz et al. 2018). With enhanced ascorbate peroxidase (APX) and class-III peroxidase (POD) tobacco plants tolerated up to 6.9 kJ m-2 d-1 biologically effective doses of UV-B radiation without major loss of photosynthetic yield in growth chambers, although cellular H2O2 concentrations increase under these conditions. In this study, we compared enzymatic and non-enzymatic leaf responses to exogenous H2O2 and to supplemental UV-B as single factors and to a combination of these two factors in order to study to what extent responses to UV-B-inducible H2O2 and directly to UV-B might overlap. Tobacco plants (*Nicotiana tabacum* L. cv. Xhanti) were grown and treated when four-weekold in plant chambers under long day conditions, 16h/8h light (120 mmol m-2 s-1 PAR)/dark, 20oC/24oC. H2O2 was applied as 100 mM water solution for three days and control plants were treated with equivalent amounts of water. Supplementary UV-B treatment (6.9 kJ m-2 d-1 b.e.) was applied for four days. Leaves acclimated to either UV-B or to the applied exogenous H2O2 as well as to the combined treatment without significant loss of photochemical yield. Chlorophyll contents were unaffected by either treatment. Adaxial leaf flavonoid indexes increased in UV-Bexposed leaves but not upon the H2O2 treatment. Non-enzymatic H2O2 neutralization showed an opposite trend and increased upon the direct ROS treatment only, indicating that H2O2-responsive non-enzymatic antioxidants are distinct from epidermal flavonoids measured as the adaxial flavonoid index. Enzymatic H2O2 neutralization, on the other hand, was more responsive to the UV-B than to the direct ROS treatment. APX increased in response to UV-B only. Total POD activity as assayed with the synthetic substrate ABTS increased after either UV-B or H2O2 treatment. However, activities of POD isoforms using phenolic compounds as substrates (such as chlorogenic acid and quercetin present in the tobacco leaves) were increased by UV-B only. SOD activity, as potential internal source of H2O2 was not responsive to either treatment. These results suggest that although UV- and H2O2-responsive antioxidant pathways partly overlap; in this model experiment oxidative stress in UV-B exposed leaves is avoided (and acclimation is achieved) via direct UV-inducible responses, rather than those triggered by UV-induced H2O2 production. Czégény Gy, Mátai A, Hideg É (2016) UV-B effects on leaves – oxidative stress and acclimation in controlled environments. Plant Sci. 248:57-63 Rácz A, Hideg É, Czégény Gy (2018) Selective responses of class III plant peroxidase isoforms to environmentally relevant UV-B doses. J. Plant Physiol. 221:101 -106 Research was funded by the National Research, Development and Innovation Office (grant number K124165) and supported by the ÚNKP-18-3 New National Excellence Program of the Ministry of Human Capacities.

> P102. Poster

Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

PHYSIOLOGICAL CHARACTERIZATION OF THE EFFECT OF UV SCREENING ON DNA DAMAGE INDUCTION AND PHOTOREPAIR IN THE GREEN MACROALGA CLADOPHORA SP. AS COMPARED TO THE NON-UV SCREENING SPECIES ULVA SP

Authors: Frauke Pescheck[1]
Presenting Author: Frauke Pescheck
1) Botanical Institute, Christian Albrechts University

This study sheds light on UV resistance mechanisms of two green macroalgae which pursue very distinct strategies to successfully co-occur in a strongly UV exposed habitat.

The Cladophorales is one of a few orders among green macroalgae that possess efficient UV screening located in their cell walls. However, the chemical identity of the responsible UV absorbing compound(s) and their absorption properties are unknown up to now. Therefore, physiological experiments were used to investigate the spectral properties of the UV screening *in vivo* in comparison to the non-UV screening green macroalga *Ulva* sp.. The apparent *in vivo* transmission spectra of the cell walls were calculated from differential chlorophyll fluorescence excitation spectra of intact thalli and isolated chloroplasts in both species. UV screening in *Cladophora* sp. was maximal around 315 nm with detectable absorption up to 400 nm (1). UV-B induced DNA damage was proportionally lowered to the extent of apparent UV screening *in vivo* in this species (2). On the other hand, a reduced rate of DNA repair by photoreactivation was observed in *Cladophora* sp. as compared to *Ulva* sp. under experimental UV-A radiation (2). Photorepair rates under light limiting conditions were quantitatively related to the lowered internal availability of photoreactivating photons in *Cladophora* sp. as compared to *Ulva* sp. (1). The ecological significance of this effect of UV screening on the balance of DNA damage induction and photorepair was modeled for sunlight. Weighted solar spectra using previously published action spectra for DNA damage induction and photoreactivation were set off against apparent *in vivo* UV transmission spectra of both species. Integration of the resulting internal effective spectra indicates that the photoprotective effect of UV screening in *Cladophora* sp. increases the DNA stability compared to *Ulva* sp. by more than a factor of 2 while *Ulva* sp. uses UV-A radiation around 25 % more efficiently for photoreactivation. Clearly, in view of UV effects on DNA integrity the advantage of UV screening in *Cladophora* sp. outweighs the better UV-A usage in *Ulva* sp. without UV screening.

References

(1) F. Pescheck, Photochem Photobiol Sci, 2019, 18, 413 -423.
(2) F. Pescheck and W. Bilger, Mar Biol, 2018, 165:132.

> P103. Poster

Symposium PLANT-4 Effect of UV light on Plants (Gareth Jenkins)

DIVERGENT ROLES OF ARABIDOPSIS RUP1 AND RUP2 AS REPRESSORS OF FLOWERING UNDER UV-B

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Presenting Author: Song Chen
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Plants have evolved a specific perception system to monitor the changing UV-B levels from sunlight in order to respond and prevent damages caused by UV-B. Arabidopsis UV RESISTANCE LOCUS8 (UVR8) was identified as the receptor for UV-B with its intrinsic tryptophan residues serving as chromophores. REPRESSOR OF UV-B PHOTOMORPHOGENESIS1 (RUP1) and RUP2 are two negative regulators that repress UVR8 function to prevent the plants from over-responding to UV-B. Present understanding of the role of UV-B signaling is largely associated with UV-B acclimation and tolerance, but additional roles are emerging. We have recently described a novel link between UVR8 photoreceptor signaling and photoperiodic flowering. Mutation of the *RUP2* gene renders the facultative long-day plant Arabidopsis into a day-neutral plant, specifically under conditions including UV-B. The wild-type RUP2 protein thus functions as a crucial repressor of UVR8-induced flowering under short day conditions. In contrast, *RUP1*, the closely related homolog of *RUP2,* apparently does not play any role in repressing floral transition under UV-B. In order to reveal the mechanisms behind the functional divergence between RUP1 and RUP2 in controlling photoperiodic flowering, we generated and studied promoter swap lines, ubiquitously RUP1 and RUP2 expressing lines, and lines expressing RUP1 and RUP2 with tissue-specific promoters. We will present our current understanding of photoperiodic flowering in the presence of UV-B.

> IL172. Invited Lecture
Symposium PLANT-5 Photosynthetic Organisms as Biofactories (Wim Vermaas)

LIGHT-CATALYZED PRODUCTION OF FATTY ACIDS AND THEIR DERIVATIVES FROM CO_2 USING CYANOBACTERIA

Authors: Shuqin Li[1], Wim Vermaas[1]
Presenting Author: Wim Vermaas
1) Arizona State University

Cyanobacteria are excellent organisms for production of excreted biofuels and green chemicals because they are photosynthetic (producing organic compounds from CO_2, water and light) and typically excrete produced compounds much more easily than other phototrophs such as algae do. Excretion of product helps to alleviate feedback inhibition of product formation and enhances the economic feasibility of the process.

We have generated a laurate-producing and -excreting strain of the cyanobacterium *Synechocystis* sp. PCC 6803 that contains a thioesterase from the plant *Umbellularia californica*, releasing the fatty acid laurate when native fatty acid biosynthesis reaches the C12 stage. This strain is efficient in producing laurate, a fatty acid that can be used as a biofuel precursor, from CO_2 that was fixed by photosynthesis. The amount of fatty acid produced, typically in the range of 0.7 mM in the medium, represents about 20% of photosynthetically fixed carbon in cells.

However, laurate is readily consumed by many heterotrophic prokaryotes. Therefore, we added a methylation step to convert laurate to the more stable and water-insoluble methyl laurate. This conversion of laurate to methyl laurate is done by a S-adenosyl methionine (SAM)-dependent enzyme. Main advantages over current biofuel products are methyl laurate's immediate application as biodiesel and its limited solubility in water, thus reducing the availability to heterotrophs in the culture and increasing the ease of harvesting. Morever, lauroyl esters have many additional applications. This approach provides a 'one-stop-shop' cyanobacterial platform that generates liquid transportation fuel from CO_2 and water with sunlight as the energy input.

The US Department of Energy funded this work (EERE grant EE0007561).

> IL173. Invited Lecture
Symposium PLANT-5 Photosynthetic Organisms as Biofactories (Wim Vermaas)

TOWARDS SUSTAINABLE PRODUCTION OF BIOFUELS BY "MILKING" OF THE CYANOBACTERIAL CELLS ENGINEERED FOR FREE FATTY ACID PRODUCTION

Authors: Tatsuo Omata[1]
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Introduction

Photosynthetic microorganisms are thought to provide a promising platform for biofuel production, but algae-based biofuel production suffers from the large energy input required to harvest and to dry the cells[1)], which makes the energy-profit ratio (EPR) smaller than 1. The EPR value can be increased by having the cells secrete the oily product out of the cells during photosynthesis; With a product-to-cell ratio of 1 (wt./wt.), an EPR value of 1.3 would be expected if the entire product is secreted. This strategy for biofuel production, *i.e.*, "milking" of algal cells[2)], requires (i) a high rate of oil production, (ii) rapid secretion of the product out of the cells, and (iii) regulation of cell growth to increase the product-to-cell ratio. Since none of these is compatible with the nature of photosynthetic microorganisms, we aimed at fulfilling the requirements by genetic engineering of cyanobacteria.

Methods and Results

To achieve milking of cyanobacterial cells for oil production, we have been improving the free fatty acid (FFA) production system reported by Liu et al.for *Synechocystis* sp. PCC 6803[3)], which attained a FFA-to-cell ratio of 0.13 (wt./wt.) with an average secretion rate of 0.44 mg L^{-1} h^{-1}. We chose *Synechococcus elongatus* PCC7942 as the material, because it was found to have an unusually high capacity of FFA synthesis, fulfilling the requirement (i) shown above[4)]. The engineered *Synechococcus* cells, however, suffered from severe photoinhibition because of over-accumulation of FFA[4,5)]. Enhancement of passive efflux[4)] and active export[6)] of FFA was shown to stabilize the cells and to improve FFA productivity. Removal of FFA from the culture medium was also effective for enhancement of the production[7)]. We thus attained production of 0.64 g FFA per L of culture in 432 h with an average secretion rate of 1.5 mg L^{-1} h^{-1}, but the FFA-to-cell ratio was still low, being 0.36[7)]. To fulfill the requirement (iii), we slowed cell growth by N limitation while enhancing FFA export. This increased the FFA secretion rate and the FFA-to-cell ratio to 1.8 mg L^{-1} h^{-1} and 0.9, respectively, but the system could be maintained only for 240 h, producing 0.45 g FFA per L of culture.

Discussion

Sustained FFA production via milking of cyanobacterial cells would be achieved by keeping the rate of FFA secretion higher than that of FFA production in the cell. Further enhancement of FFA export out of the cell and its removal from the culture medium are crucial.

References

1) Lardon, L. et al. (2009) *Environ. Sci. Technol.* 43: 6475–6481
2) Ramachandra, T.V. et al. (2009) *Ind. Eng. Chem. Res.* 48: 8769–8788
3) Liu, X. et al. (2011) *Proc. Natl. Acad. Sci. USA* 108: 6899-6904
4) Kato, A. et al. (2016) *Biotechnol. Biofuels* 9: 91,
5) Takatani, N. et al. (2015) *Plant Cell Physiol.* 56: 1608-1615
6) Kato, A. et al. (2015) *Plant Cell Physiol.* 56: 2467-2477
7) Kato, A. et al. (2017) *Biotechnol. Biofuels* 10: 141

> IL174. Invited Lecture
Symposium PLANT-5 Photosynthetic Organisms as Biofactories (Wim Vermaas)

TARGETED GENE REPRESSION (CRISPRI) APPLIED TO CYANOBACTERIA FOR RAPID AND MULTIPLEX METABOLIC ENGINEERING

Authors: Paul Hudson[KTH R]
Presenting Author: Paul Hudson
1) KTH Royal Institute of Technology

Introduction
A desirable trait for industrial cyanobacteria strain is the ability switch between two metabolic states: biomass accumulation to a desired cell density followed by product synthesis, each with a high rate of CO_2 fixation. One way to effect such a metabolic switch is through targeted gene repression using CRISPR interference (CRISPRi). CRISPRi utilizes inducible expression of the dCas9 repressor and a sequence specific guide RNA (sgRNA), which combine to block transcription of a target gene. Furthermore, since the sgRNA is small (>100 nt), pools of sgRNAs can be easily synthesized and the resulting CRISPRi "libraries," can be screened for improved productivities. CRISPRi is thus a valuable tool for rapidly screening metabolic engineering strategies.

Methods
CRISPRi was adapted for use in the model cyanobacteria Synechocystis [1]. In one application, expression of the central metabolic enzyme citrate synthase was repressed in a Synechocystis strain producing lactic acid [2]. In a second application, gene repression "libraries" were created (12,000 sgRNAs) and screened for increased biomass or lactate productivity [3].

Results and Discussion
CRISPRi repression of citrate synthase repressed cell growth while incoming CO2 was diverted to lactate at over 75% yield. However, specific CO2 fixation rate decreased after several days. A CRISPRi library was screened on the basis of both cell growth and production of l-lactate. Several clones were found that showed increased growth rate (up to 15% increase). Transcriptomics analysis of these strains showed common gene regulation patterns. Using a previously established droplet microfluidics sorting setup [3], we were able to isolate clones that produced more lactic acid.

Conclusions
The "growth arrest" of cyanobacteria allows for high CO_2 flux to product over a period of several days. However, the connection between reduced growth rate and reduced CO_2 uptake must be elucidated and de-regulated. CRISPRi is a useful tool for testing single or combinations of gene repression strategies. Furthermore, the presence of several faster-growing clones in the CRISPRi library show that gene expression in "wild-type" Synechocystis is suboptimal for fast growth in constant light.

References

[1] Yao et al., 2016. Multiple Gene Repression in Cyanobacteria Using CRISPRi. ACS Synthetic Biology 5, 207-212
[2] Shabestary et al., 2018. Targeted Repression of Essential Genes To Arrest Growth and Increase Carbon Partitioning and Biofuel Titers in Cyanobacteria. ACS Synthetic Biology 7, 1669-1675
[3] Hammar et al., 2015. Single-cell screening of photosynthetic growth and lactate production by cyanobacteria. Biotechnology for Biofuels 8, 193-201

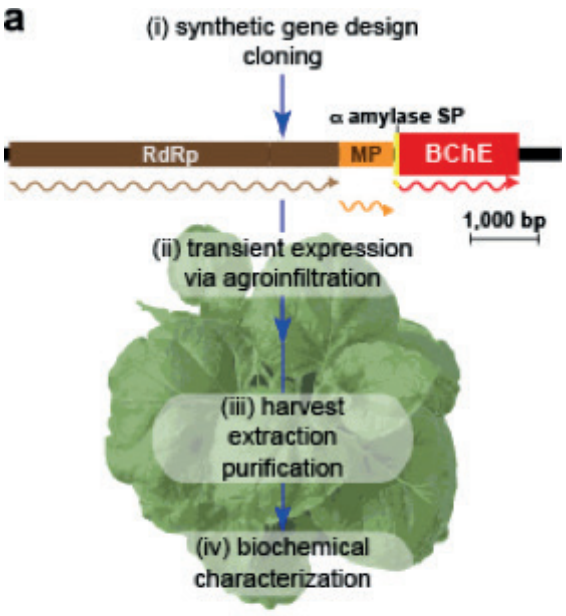
> IL175. Invited Lecture

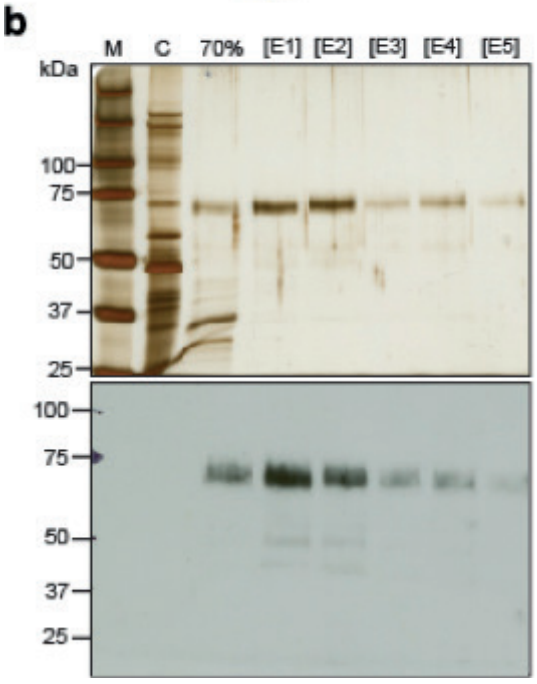
Symposium PLANT-5 Photosynthetic Organisms as Biofactories (Wim Vermaas)

A PLANT-DERIVED COCAINE HYDROLASE PREVENTS COCAINE OVERDOSE LETHALITY AND ATTENUATES COCAINE-INDUCED DRUG SEEKING BEHAVIOR

Authors: Katherine Larrimore[1], Latha Kannan[1], Player Kendle[1], Tameem Jamal[1], Matthew Barcus[1], Kathryn Stefanko[1], Jacquelyn Kilbourne[1], Stephen Brimijoin[2], Chang-Guo Zhan[3], Janet Neisewander[1], Tsafrir Mor[1]
Presenting Author: Tsafrir Mor
1) Arizona State University 2) Mayo Clinic 3) University of Kentucky

Cocaine use disorders include short-term and acute pathologies (e.g. overdose) and long-term and chronic disorders (e.g. intractable addiction and post-abstinence relapse) that affect millions of people around the world. There is currently no available treatment that can effectively reduce morbidity and mortality associated with cocaine overdose or that can effectively prevent relapse in recovering addicts. One approach recently developed to treat these problems is the use of enzymes that can break down the active cocaine molecule into inactive metabolites. In particular, rational design and site directed mutagenesis transformed human serum recombinant butyrylcholinesterase (BChE) into an efficient cocaine hydrolase with drastically improved catalytic efficiency toward (-)-cocaine. Plants can serve as a safe, cost-effective, and easily scalable production system for a range of recombinant BChE variants. Here we demonstrate that a **P**lant-derived form of the **Coc**aine **S**uper **H**ydrolase (A199S/F227A/S287G/A328W/Y332G), which we call PCocSH, protects mice from cocaine overdose, counters the lethal effects of acute cocaine overdose, and prevents reinstatement of extinguished drug-seeking behavior in mice that underwent place conditioning with cocaine. These results demonstrate that the novel PCocSH enzyme might serve as an effective therapeutic forcocaine use disorders in a clinical setting.





> IL176. Invited Lecture
Symposium PLANT-5 Photosynthetic Organisms as Biofactories (Wim Vermaas)

PHOTOSYNTHETIC MICROBES AS CELL FACTORIES FOR SUSTAINABLE BIOPRODUCTION

Authors: Himadri Pakrasi[Washi]
Presenting Author: Himadri Pakrasi
1) Washington University

Photosynthetic microorganisms, and especially cyanobacteria, hold great promise as cell factories for sustainable production of bulk and specialty chemicals as well as nutritional compounds. While these organisms may be more difficult to work with as "chassis" strains for synthetic biology than certain heterotrophs, the unique advantages of autotrophs in biotechnology applications as well as the scientific importance of improved understanding of photosynthesis warrant the development of these organisms into systems akin to "green *E. coli*". The commonly used photosynthetic microbial organisms grow significantly slower than industrially relevant heterotrophic microbes. During recent years, we have identified a cyanobacterium that grow as fast as yeast, while using only light and CO_2 as the principal feedstocks. The potentials of such fast-growing organisms as autotrophic cell factories will be discussed.

> IL177. Invited Lecture
Symposium PLANT-5 Photosynthetic Organisms as Biofactories (Wim Vermaas)

MICROALGAE AS SUSTAINABLE PHOTOSYNTHETIC GREEN CELL FACTORIES FOR THE SYNTHETIC PRODUCTION OF HYDROCARBONS

Authors: Olaf Kruse[1]
Presenting Author: Olaf Kruse
1) Bielefeld University, Center for Biotechnology CeBiTec

Introduction

Microalgae are capable of efficiently converting inorganic CO_2 with the help of sunlight energy and water splitting into organic biomass, which is composed of energy-rich carbon-based compounds. Efficient photon energy conversion into bio-products of interest requires the understanding of the regulation of light energy conversion mechanisms, as well as the availability of molecular tools for the generation of mutants with enhanced efficiency as green cell factories.

Results

The design of synthetic constructs for efficient gene/protein expression and pathway engineering, performed with the microalga *Chlamydomonas reinhardtii* for the synthesis of a variety of terpenes, has been achieved by developing new specific molecular tools. These tools include a strategy for enhanced transformation efficiencies by the targeted integration of introns[1] and the development of a new molecular tool kit for gene transformation and vector shuttle systems[2]. By applying these new tools, we successfully engineered microalgae for the production of a variety of terpenes with a specific focus on diterpenes[3].

References

1. Baier, T., Wichmann, J., Kruse, O. & Lauersen, K. J. (2018) Intron-containing algal transgenes mediate efficient recombinant gene expression in the green microalga *Chlamydomonas reinhardtii*. *Nucleid Acids Res* 46: 6909-6919
2. Crozet, P., et al. (2018) Birth of a Photosynthetic Chassis: A MoClo Toolkit Enabling Synthetic Biology in the Microalga *Chlamydomonas reinhardtii*. *ACS Synth. Biol.* 7(9): 2074-2086
3. Lauersen, K.J., Wichmann, J., Baier, T., Kampranisb, S.C., Pateraki, I., Lindberg Møller, B. & Kruse, O. (2018) Phototrophic production of heterologous diterpenoids and a hydroxyfunctionalized derivative from *Chlamydomonas reinhardtii*. *Metabolic Eng*. 49: 116-127

> OC087. Oral Communication
Symposium PLANT-5 Photosynthetic Organisms as Biofactories (Wim Vermaas)

PURPLE BACTERIA & SALINE PHOTO-BIOELECTROCHEMICAL SYSTEMS: ELUCIDATING SALT ADAPTATION MECHANISMS

Authors: Matteo Grattieri[1], Erin Gaffney[1], Shelley Minteer[1]
Presenting Author: Matteo Grattieri
1) Departments of Chemistry and Materials Science & Engineering, University of Utah

The combination of photosynthetic biomaterials with an electrode surface is at the basis of the fascinating field of photo-bioelectrochemistry. These systems allow for the conversion of solar energy into electrical current,[1,2] opening for the development of photobioelectrochemical sensors for online monitoring of toxic compounds in water environments.[3] However, the on field application of such biosensors would result in the exposition of biomaterials to changing environmental conditions, making critical the use of versatile organisms capable to tolerate shock events.

Based on the presented challenge, our research efforts focused on the use of purple bacteria, photosynthetic organisms characterized by extremely versatile metabolisms. Specifically, *Rhodobacter capsulatus* (*R. capsulatus*) has a very effective anaerobic photoheterotrophic metabolism, which could be utilized to monitor the presence of contaminant in water. However, the extracellular electron transfer process (EET) of *R. capsulatus* with an electrode surface is challenging due to its redox active center being buried inside the thick cellular membrane. Our group has recently clarified the quinone-mediated extracellular electron transfer process between *R. capsulatus* cells and a carbon electrode,[4] and studies are undergoing to further enhance its EET. Herein, the importance of clarifying salt adaptation mechanisms of *R. capsulatus* for application in water samples with changing salinity will be discussed. We will introduce how increasing salinity affects bioelectrocatalytic performance of *R. capsulatus*, based on cyclic voltammetry and chronoamperometric studies. Cell transfers and prolonged exposure to increasing salt concentrations allowed bacterial adaptation to the environment, improving the photo-bioelectrochemical performance in highly saline solution (22 gL^{-1} NaCl).[5] The contribution of the *R. capsulatus* gene transfer agent, as well as quorum sensing autoinducers on cells adaptation to increasing salt content will be presented, as well as their influence on the photo-bioelectrochemical performance. Furthermore, RNA sequencing was performed to monitor changes in genes expression after the exposure to saline conditions.

Our results shows that the elucidation of salt adaptation mechanisms provides critical insights for the enhancement of photo-bioelectrochemical performance, setting the on field application of these systems a step closer.

The authors declare no competing financial interest.

References

[1] M. Grattieri, S.D. Minteer. *Nature Energy* **2018**, 3: 8
[2] K. Hasan, V. Grippo, E. Sperling, M.A. Packer, D. Leech, L. Gorton. *ChemElectroChem* **2017**, 4: 412
[3] M. Tucci, M. Grattieri, A. Schievano, P. Cristiani, S.D. Minteer. *Electrochimica Acta* **2019**, 302: 102
[4] M. Grattieri, Z. Rhodes, D.P. Hickey, K. Beaver, S.D. Minteer. *ACS Catalysis* **2019**, 9: 867
[5] M. Grattieri, K. Beaver, E.M. Gaffney, S.D. Minteer. *Faraday Discussions* **2019**, DOI: 10.1039/C8FD00160J

> P104. Poster

Symposium PLANT-5 Photosynthetic Organisms as Biofactories (Wim Vermaas)

HOW TO IMAGE CARBON DYNAMICS OF PHOTOSYNTHESIS AND PHOTOSYNTHETIC PRODUCTS

Authors: Keisuke Kurita[1], Yuta Miyoshi[1], Yong-Gen Yin[1], Satomi Ishii[1], Nobuo Suzui[1], Naoki Kawachi[1]
Presenting Author: Naoki Kawachi
1) National Institutes for Quantum and Radiological Science and Technology

Radionuclide imaging technologies have opened up experimental opportunities for biological research. However, the conventional measurement tools used in plant science are invasive and require calibration by statistical analysis over a large number of test plants. RI imaging is one of the most powerful tools for conducting research on the distribution and translocation nutrition of water, nitrogen, mineral nutrients, etc., and environmental pollutants in plants, noninvasively. For analysis of carbon kinetics in a plant body, it is possible with the positron-emitting radioisotope C-11, which has a short half-life, and positron imaging systems. The carbon kinetics makes it a strong potential candidate for application to the analysis of physiologies involved in photosynthesis and photoassimilate translocation. The C-11 imaging approach has been used for real-time and quantitative video imaging of tracer dynamics during carbon fixation, photosynthesis, and photoassimilate translocation. In this paper, we describe the latest method to image the dynamics of C-11 compounds in the plant body using RI imaging method and discuss its applicability to investigations of the kinetics of carbon nutrients during photosynthesis and photoassimilate translocation and unloading. Elucidation of the carbon kinetics in a plant body clearly leads to agricultural study on the growth and development of grains and fruits.

> IL180. Invited Lecture
Symposium PLANT-6 Artificial Photosynthesis (Sebastiano Campagna)

HYBRID PHOTOSYNTHETIC ENZYMES AS PHOTOACTIVE SOFT MATERIALS

Authors: Massimo Trotta[1,2]
Presenting Author: Massimo Trotta
1) Istituto per i Processi Chimico Fisici - Consiglio Nazionale delle Ricerche 2) Dipartimento di Chimica - Università di Bari

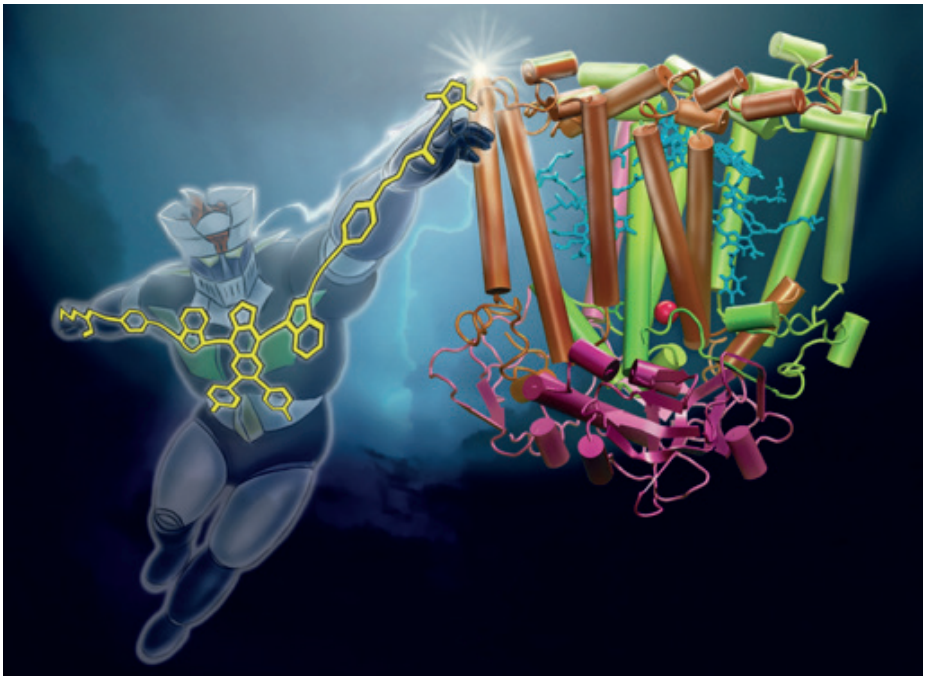
The complexity of the natural photosynthetic systems is difficult to reproduce *in vitro*; however, complexity is inherently associated to the efficiency of the living multienzyme character of photosynthesis and any biomimetic attempts must cope with this stringent requirement.

In this regard, we have designed and assembled efficient organic-biological hybrid systems formed by small to medium size organics molecules responsible of a given specific role and the photoenzyme responsible for energy transduction in photosynthetic organisms.

Applications of photoresponsive enzymes as soft photoconverting material in different environment will be presented to show drawbacks, limitations and potentials of such hybrid systems, along with some future interesting developments.

References

1. *Enhancing light harvesting capability of the photosynthetic reaction centre by a tailored molecular fluorophore*. 2012 ***Angewandte Chemie Int. Ed.*** **51**, 11019.
2. *Synthetic Antenna Functioning As Light Harvester in the Whole Visible Region for Enhanced Hybrid Photosynthetic Reaction Centers* 2016 ***Bioconj. Chemistry*** **27**, 1614.
3. *Highly oriented photosynthetic reaction centers generate a proton gradient in synthetic protocells* 2017 **PNAS 114**, 3837.
4. *Functional Enzymes in Nonaqueous Environment: the Case of Photosynthetic reaction centers in Deep Eutectic Solvents,* 2017 **ACS Sustainable Chem. Eng.**, **5**, 7768.
5. *A highly efficient heptamethine cyanine antenna for photosynthetic Reaction Center: From chemical design to ultrafast energy transfer investigation of the hybrid system*, 2019 **BBA-Bioenergetics, 1860** 350-359.
6. *Photonics and Optoelectronics with Bacteria: Making Materials from Photosynthetic Microorganisms.* ***Adv. Func. Materials****,* 2019 in press (doi.org/10.1002/adfm.201805521)



> IL178. Invited Lecture
Symposium PLANT-6 Artificial Photosynthesis (Sebastiano Campagna)

POLYPYRIDINE TRANSITION METAL COMPLEXES AS HOMOGENEOUS CATALYSTS FOR ARTIFICIAL PHOTOSYNTHESIS

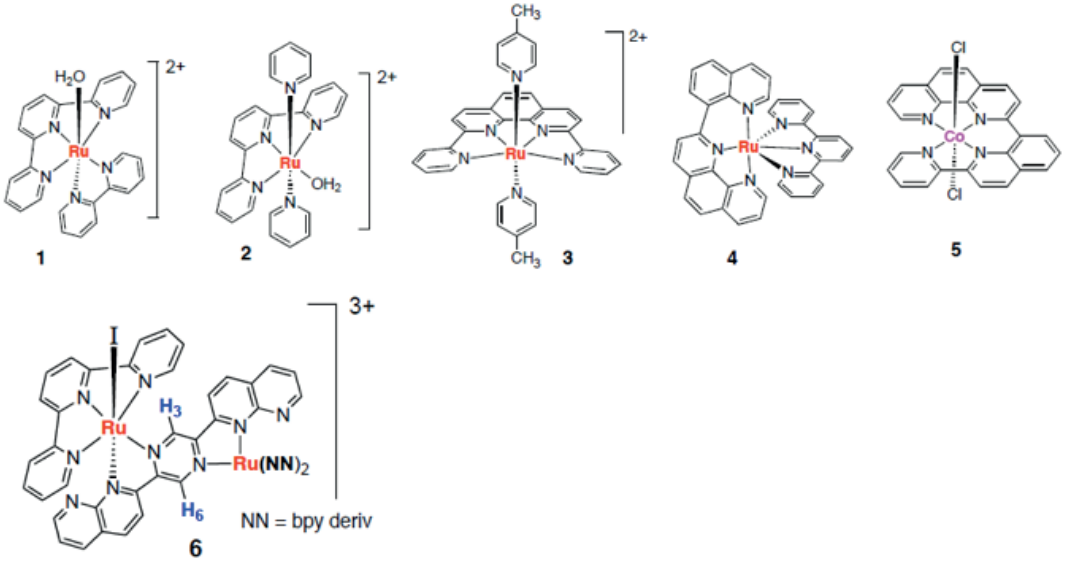
Authors: Randolph Thummel[1], Liubov Lifshits[1], Lanka Wickramasinghe[1], Elamparuthi Ramasamy[1]
Presenting Author: Randolph Thummel
1) Department of Chemistry, University of Houston

In order to produce hydrogen from water in a photocatalytic process, one must also produce oxygen. Compared to water reduction, the oxidation of water has long been considered the more challenging process since it involves the loss of four electrons and the combination of two water molecules. Early work suggested that a dinuclear metal complex would be best suited to this process because it could provide the required orientation of two water molecules such that an O=O bond might form. In 2005, using an approach driven by ligand synthesis, we discovered that mononuclear Ru(II) complexes such as**1** and **2** could, in fact, catalyze water oxidation and a mechanism involving water attack on an intermediate Ru=O species was suggested. Surprisingly the complex **3**, that did not involve a water bound to Ru(II), was even more effective in catalysis. We have proposed that upon oxidation of the metal center from Ru(II) to Ru(IV), a water molecule attacks in the equatorial plane, expanding the coordination number to seven. We have subsequently discovered that slight changes in the steric environment around the metal center can have a profound influence on reactivity such that complex **4** serves as an effective catalyst while the closely related $[Ru(tpy)_2]^{2+}$ (tpy = 2,2';6',2"-terpyridine) is completely unreactive.[1]

In recent work, these catalysts have been incorporated into dyad assemblies such as **6** where a Ru(II)-based photosensitizer can drive the oxidation process with light. To function successfully the $Ru(NN)_2$ sensitizer must have an excited state reduction potential that is greater than the first oxidation potential of the catalyst portion of the dyad. More recently we have prepared a Co(II) complex **5** involving the 5-6-5 chelating ligand ppq. This complex is an efficient proton reduction catalyst while the analogous Fe(III) ppq complex, as a m-oxo-bridged dimer, is very effective at water oxidation. For both oxidation and reduction to function, sacrificial reagents are needed (Ce^{IV} or $S_2O_8^{2-}$ for oxidation and ascorbic acid for reduction). If both redox reactions can be photoactivated simultaneously, the sacrificial reagents may no longer be needed. Recently we have examined a wide range of potential catalysts involving mono-anionic tridentate and tetradentate ligands and these new systems will be presented.

Reference

1. Tong, L.; Thummel, R. P. *Chemical Science,* **2016**, *7,* 6591-6603



> IL181. Invited Lecture
Symposium PLANT-6 Artificial Photosynthesis (Sebastiano Campagna)

MULTICOMPONENT METAL POLYPYRIDINE COMPLEXES FOR MOLECULAR-BASED ARTIFICIAL PHOTOSYNTHESIS

Authors: Sebastiano Campagna[1], Scolastica Serroni[1], Fausto Puntoriero[1], Giuseppina La Ganga[1], Francesco Nastasi[1]
Presenting Author: Sebastiano Campagna
1) University of Messina, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali

According to a bio-mimetic approach, molecular-based artificial photosynthesis requires the design of several components, each of them a supramolecular system by itself, structurally-organized and functionally-integrated. In such integrated artificial assemblies, photons and electrons have to be elaborated in well-organized fashions, and all the processes must be orchestrated in the dimension of space, energy, and time (1). Within this framework, multinuclear Ru(II) complexes have proved to be of a large interest (2).

Here we present some results, based on multicomponent Ru(II) compounds, recently obtained by our group related to (i) artificial light-harvesting antenna systems (role: absorbing light and converting it into electronic energy, which can be funneled to specific sites of the assemblies) (3); (ii) charge separation systems (role: to use the electronic energy collected by the antennae to perform charge separation, that is to transform electronic energy into redox energy) (4); (iii) integrated antenna and catalysts for water oxidation. We acknowledge support from MAECI (Progetti di Grande Rilevanza Italia-Giappone).

References

1) N. Armaroli, V. Balzani, *Energy for a Sustainable World*, Wiley, **2014**.
2) V. Balzani, S. Campagna, G. Denti, A. Juris, S. Serroni, M. Venturi, *Acc. Chem. Res.*, **1998**, *31*, 26.
3) A. Arrigo, F. Puntoriero, G. La Ganga, S. Campagna, M. Burian, S. Bernstorff, H. Amenitsch, *Chem*, **2017**, *2*, 494.
4) M. Natali, S. Campagna, F. Scandola, *Chem. Soc. Rev.,* **2014**, *43*, 4005

> IL183. Invited Lecture
Symposium PLANT-6 Artificial Photosynthesis (Sebastiano Campagna)

MOLECULAR PHOTOCATALYSIS TOWARDS SOLAR WATER SPLITTING AND CARBON DIOXIDE REDUCTION

Authors: Ken Sakai[1]
Presenting Author: Ken Sakai
1) Department of Chemistry, Faculty of Science, International Institute for Carbon-Neutral Energy Research, and Center for Molceular Systems of Kyushu University, Motooka 744, Nishi-ku, Fukuoka 819-0395, Japan

Over the past decade, our group has focused on the studies of transition-metal-based molecular systems relevant to the development of artificial photosynthetic molecular devices. The targets of our research involve the studies on (i) water oxidation catalysis in order to uptake protons and electrons required for fuels generation, (ii) catalytic water or CO_2 reduction into sustainable fuels (*i.e.*, H_2, CO, etc.), (iii) artificial light-harvesting systems towards the effective charge separation and/or migration, and (iv) molecular- and instrumental-level chemical engineering by making hybrid molecular and/or heterogeneous systems using multiple key components. Deeper insights into the mechanism of reaction of interest are always greatly appreciated for the sake of inspiring the rational design strategies towards the more desirable/efficient systems in promoting all relevant processes. In this context, substantial efforts have been devoted to more carefully study the reaction kinetics and equilibria in solution that are relevant to each topic. Various spectrophotometric, electrochemical, and photochemical techniques have been adopted to better understand the mechanistic aspects relevant to all of our systems. Some of the reaction steps of interest are not observable by any experimental techniques, and must be discussed on the basis of our DFT results, which have also greatly helped us understand the mechanism of reactions. Importantly, one of our findings is that, in any catalysis, the reactivity of metal(s) can be rationally tuned by use of redox active ligands that are more or less hybridized with metal(s) in their orbitals. Such issues are often involved in our discussion. One of our interests has concentrated on the molecular Pt-catalyzed hydrogen evolution reactions and their application to fabricate photosensitizer-catalyst hybrid molecular devices [1-3]. Our recent kinetic and electrochemical studies evidence the formation of a hydridodiplatinum(II,III) intermediate when H_2 evolution is catalyzed by a simple mononuclear Pt(bpy)Cl_2 derivative, which is also rationalized by our DFT results. Our studies have also provided new aspects on photo-induced multi-charge separation [4], near-infrared-driven water reduction [5], water oxidation catalysis using various transition metal complexes [6,7], non-precious metal based H_2 evolution catalysis [8], and photoelectrochemical cells for the overall water splitting [9].

References

1. Ozawa, H.; Haga, M.; Sakai, K. *J. Am. Chem. Soc.* **2006**, *128*, 4926-4927.
2. Sakai, K.; Ozawa, H. *Coord. Chem. Rev.* **2007**, *251*, 2753-2766.
3. Kitamoto, K.; Sakai, K. *Angew. Chem. Int. Ed.* **2014**, *53*, 4618-4622.
4. Kitamoto, K.; Sakai, K. *Chem. Eur. J.* **2016**, *35*, 12381-12390.
5. Tsuji, Y.; Yamamoto, K.; Yamauchi, K.; Sakai, K. *Angew. Chem. Int. Ed.* **2018**, *57*, 208-212.
6. Nakazono, T.; Sakai, K. *Dalton Trans.* **2016**, *45*, 12649-12652.
7. Parent, A.R.; Nakazono, T.; Tsubonouchi, Y.; Taira, N.; Sakai, K. *Adv. Inorg. Chem.* **2019**, in press.
8. Koshiba, K.; Yamauchi, K.; Sakai, K. *ChemElectroChem* **2019**, published online.
9. Morita, K.; Sakai, K.; Ozawa, H. *ACS Appl. Energy Mater.* **2019**, *2*, 987-992.

> IL182. Invited Lecture
Symposium PLANT-6 Artificial Photosynthesis (Sebastiano Campagna)

SUPRAMOLECULAR ARCHITECTURES FOR ARTIFICIAL PHOTOSYNTHESIS

Authors: Marcella Bonchio[1]
Presenting Author: Marcella Bonchio
1) ITM-CNR and University of Padova

Introduction

The O_2 necessary for our aerobic life is produced by the photocatalytic cleavage of the extremely stable H_2O bonds. Making oxygen is exceptionally difficult and lethal for any biological factory, which calls out a continuous self-repair cycle during oxygenic photosynthesis. Indeed, and despite the vast bio-diversity footprint, just one specialized protein complex is used by Nature as the H_2O-photolyzer: photosystem II (PSII). Man-made systems are still far from replicating the complexity of PSII. High resolution imaging of the PSII "core" complex shows the ideal co-localization of multi-chromophore Light Harvesting antennas with the functional Reaction Center (LH-RC). This notion fits the so-called "quantasome" model, focusing on the functional ensemble rather than on the individual tasks of each component. Our results overcome the classical "photo-dyad" model, based on a donor-acceptor binary combination, and reach out to the quantasome archetype.

Results and Discussion

Here we report the self-assembly of multi-perylenebisimide chromophores (PBI) shaped to function by interaction with a polyoxometalate water oxidation catalyst (Ru_4POM). The resulting $[PBI]_5Ru_4POM$ complex is identified as the minimal photosynthetic unit, formed both in solution and on photoelectrodes, showing a "quantasome"-like behavior: (i) a red-shifted, light harvesting efficiency (LHE>40%), (ii) favorable exciton accumulation and negligible excimeric loss; (iii) a robust amphiphilic structure; (iv) dynamic aggregation into large 2D-paracrystalline domains. Our results include the X-ray diffraction analysis of a dense, quasi-hexagonal packing of the PBI-quantasome motif ($\{[PBI]_5Ru_4POM\}_n$), showing a striking analogy with the coexistence of fluid-to-crystalline phases in the native photosynthetic membrane. Photoexcitation of the PBI-quantasome triggers one of the highest driving force for photo-induced electron transfer applied so far yielding ultra-fast charge separation in the ps timescale, and winning over recombination by ca. two orders of magnitude. Such a long lived charge-separated species, is likely favored by electron delocalization along the π-backbone of the multi-PBI arrangement.

Conclusions

It turns out that photoanodes integrating the PBI-quantasome evolve oxygen with quantitative faradaic yield, and a peak quantum efficiency using "green" photons (λ> 500 nm) similar to PSII-bioelectrodes. The modularity of the building blocks, the simplicity of the non-covalent chemistry and the biomimetic appeal of the quantasome approach, offer a unique opportunity for innovation in Artificial Photosynthesis

References

1) Sartorel, A., Carraro, M., Toma, F. M., Prato, M. & Bonchio, M. Shaping the beating heart of artificial photosynthesis: oxygenic metal oxide nano-clusters. *Energy Environ. Sci.* **5,** 5592 (2012)
2) Bonchio, M., Sartorel, A., Prato, M. et al. Hierarchical organization of perylene bisimides and polyoxometalates for photo-assisted water oxidation. *Nature Chemistry* **11**, 146–153 (2019)

> IL179. Invited Lecture
Symposium PLANT-6 Artificial Photosynthesis (Sebastiano Campagna)

SEMI-ARTIFICIAL PHOTOSYNTHESIS: A PLATFORM TO STUDY AND REWIRE PHOTOSYNTHESIS

Authors: Jenny Zhang[Unive]
Presenting Author: Jenny Zhang
1) University of Cambridge

The ability to harness sunlight for performing large scale conversion of abundant/cheap materials to useful chemicals and fuels, in what is known as artificial photosynthesis, would pave the way for cleaner and more renewable energy sources in the future. Nature has already achieved this feat billions of years ago through photosynthesis; however, the process was evolved for survival and not efficiency. The emerging field of semi-artificial photosynthesis aims to combine the strengths of materials chemistry with synthetic biology to explore novel pathways for efficient solar-to-chemical conversion, which are otherwise inaccessible to either field alone.[1]

Here, I will describe how the water oxidation enzyme, photosystem II (PSII), thylakoid membranes, and live photosynthetic cells can be wired to high surface area electrodes to harness electrons stemming from photosynthesis for driving solar fuel conversion reactions and to interrogate biological activity.[2-4] Lessons gained from these studies may inform future developments in biophotovoltaics, bio-energy conversion technologies, and chemical biology tools for studying photosynthesis.

References

1. Kornienko, N.; Zhang, J. Z.; Sakimoto, K. K.; Yang, P.; Reisner, E. *Nature Nanotech.*, 2018, 13, 890−899.
2. Kato, M.; Zhang, J. Z.; Paul, N.; Reisner, E. *Chem. Soc. Rev.*, 2014, 43, 6485–6497.
3. Zhang, J. Z.; Paul, N.; Sokol, K. P.; Romero, E.; Grondelle, R-V.; Reisner, E. *Nature Chem. Bio*. 2016, 12, 1046–1052.
4. Zhang, J. Z.; Bombelli, P.,; Sokol, K.; Fantuzzi, A.; Rutherford, A. W.; Howe, C.; Reisner, E., 2017, *J. Am. Chem. Soc.*, 2018, 140, 6-9.

> IL184. Invited Lecture
Symposium PLANT-6 Artificial Photosynthesis (Sebastiano Campagna)

PROTON COUPLED ELECTRON TRANSFER IN ARTIFICIAL PHOTOSYNTHETIC CONSTRUCTS

Authors: Thomas A. Moore[1], Ana L. Moore[1], S. Jimena Mora[1], Emmanuel Odella[1], Brian Wadsworth[1], Gary F. Moore[1], Devens Gust[1]
Presenting Author: Thomas A. Moore
1) Center for Bioenergy and Photosynthesis, School of Molecular Sciences, Arizona State University

In photosystem II, the oxidation of Yz by P680•+ occurs with the transfer of the phenolic proton to the imidazole group of a hydrogen-bonded histidine (His190).1 This PCET process serves as a redox relay between P680•+ and the water oxidizing catalyst. Benzimidazole-phenol (BIP) and several of its derivatives serve as models of His190 and the phenol models Yz. With a simple BIP, upon electrochemical oxidation of the phenol proton transfers from the phenol to the imidazole; this is known as a one-electron, one-proton transfer (E1PT) process. A one-electron two-proton transfer, known as an E2PT process, has been shown to take place in amino-substituted BIPs upon the electrochemical oxidation of the phenol.2 In this case, a decrease in the redox potential of the phenoxyl radical/phenol couple by ~300 mV was observed.2 In order to reduce this loss in redox potential, alternative models of the Yz-His190 pair, BIP derivatives with imine substituents having lower pKa's were synthesized and results indicate that the phenol oxidation in these derivatives occurs at ~300 mV higher potential than in the amino-BIPs. Protonation of the benzimidazole, indicating an E1PT process and protonation of the imine, indicating an E2PT process can be unambiguously detected by infrared spectroelectrochemistry (IRSEC) upon oxidation of the phenol.3 A series of BIP derivatives having additional benzimidazoles were synthesized to investigate proton transfers characteristic of a Grotthuss-type proton wire operating in an H-bond network. These constructs demonstrated multiple proton translocation processes upon electrochemical oxidation of the phenol. By attaching a high potential porphyrin derivative to these BIPs, the dynamics of electron and proton transfer can be studied using ultrafast VIS pump IR probe and 2DEV techniques developed by Graham Fleming. Our preliminary results addressing questions such as concerted vs. stepwise mechanisms in these PCET processes will be presented. (Supported by a grant of the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences).

References

1. S. J. Mora, E. Odella, G. F. Moore, D. Gust, T. A. Moore, and A. L. Moore, Acc. Chem. Res. 2018, 51, 445–453.
2. M. T. Huynh, S. J. Mora, M. Villalba, M. E. Tejeda-Ferrari, P. A. Liddell, B. R. Cherry, A-L. Teillout, C. W. Machan, C. P. Kubiak, D. Gust, T. A. Moore, S. Hammes-Schiffer and A. L. Moore, ACS Cent. Sci. 2017, 3, 372–380.
3. E. Odella, S. J. Mora, B. L. Wadsworth, M. T. Huynh, J. J. Goings, P. A. Liddell, T. L. Groy, M. Gervaldo, L. E. Sereno, D. Gust, T. A. Moore, G. F. Moore, S. Hammes-Schiffer and A. L. Moore, J. Am. Chem. Soc. 2018, 140, 15450–15460.

> OC088. Oral Communication
Symposium PLANT-6 Artificial Photosynthesis (Sebastiano Campagna)

PHOTOCATALYTIC REDUCTION OF CO2 USING NOVEL SUPRAMOLECULAR RU(II)-RE(I) COMPLEXES AND A NAD(P)H MODEL COMPOUND AND A BENZOIMIDAZOLE DERIVATIVE AS ELECTRON DONORS.

Authors: Ambra Maria Cancelliere[1], Fausto Puntoriero[1], Francesco Nastasi[1], Osamu Ishitani[2], Sebastiano Campagna[1]
Presenting Author: Ambra Maria Cancelliere
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The increasing amount of CO_2 in the atmosphere represents a serious problem causing global warming and greenhouse effect.[1] Furthermore, the shortage of fossil fuels makes necessary to find a sustainable energy source like solar light. For solving these serious problems, artificial photosynthesis systems based on multiple subunits (photosensitizer and catalyst) are widely studied to convert CO_2 into useful and energy-rich compound like CO and HCOOH using solar light.[2] CO can be converted into liquid hydrocarbons and HCOOH can used as H_2 carrier because it is liquid at room temperature and easily storable. About that, the Re(I) complexes show high catalytic efficiency and selectivity for CO formation.[3]

To this goal, we designed and synthetized multinuclear systems for CO_2 photocatalytic reduction. These novel complexes, based on Ru(II) and Re(I), have been obtained via multi-step synthesis. In these molecules the light-harvesting subunit (photosensitizer, Ru based) and the catalyst subunit (Re based) are connected by a novel bridging ligand. Their catalytic abilities are widely investigated using two different kind of sacrificial agents and the products of the photocatalysis are quantified by gas chromatography and capillary electrophoresis.

These complexes act, effectively, as photocatalysts for CO_2 reduction, leading to CO formation with good efficiency.

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References

[1] T. R. Karl, K. E. Treberth, *Science*, **2003**, *302*, 1719.
[2] Y. Tamaki, O. Ishitani, *ACS Catal.*, **2017**, *7*, 3394.
[3] K. Koike, H. Hori, M. Ishizuka, J. R. Westwell, K. Takeuchi, T. Ibusuki, K. Enjouji, H. Konno, K. Sakamoto, O. Ishitani, *Organometallics*, **1997**, *16*, 5724 - 5729.

> OC089. Oral Communication
Symposium PLANT-6 Artificial Photosynthesis (Sebastiano Campagna)

SEMI-ARTIFICIAL PHOTOSYNTHESIS

Authors: Nicolas Plumere[1]
Presenting Author: Nicolas Plumere
1) Ruhr-Universtity Bochum

The integration of photosynthetic proteins in biophotoelectrodes is envisioned for the production of electricity[1] or chemical fuels. Redox hydrogels proved particularly suitable as matrices for the immobilization and electrical contacting of photosynthetic proteins to electrodes. We tuned the redox potentials of the electron relays and the properties of the polymeric supporting matrix to enable benchmark photocurrent densities (300 μA cm^{-2} for PS1[2] and up to 400 μA cm^{-2} for PS2 [3]) at low overpotential [4]. In analogy to the working principle of dye sensitized solar cells, an important feature of biohybrid solar cells for conversion of light to electricity is the charge carriers needed for collection of the high-energy electron from the photosystem [5]. The main limitation in energy conversion efficiency is the recombination of this charge carrier at the photoelectrode, a process that decreases both the photocurrent and the open circuit voltage. Moreover, this charge recombination process is suspected to induce degradation of the photosynthetic protein [6]. We demonstrate that the hydrogel film properties as well as the electrode surface chemistry can be tuned to minimize the various charge recombination pathways. In Addition, photodegradation directly correlates with the generation of reactive oxygen species [7]. To avoid degradation of PS1 during illumination and hence to enhance the long-term stability, the operation of biophotocathodes under anaerobic conditions is advantageous.

References

[1] N. Plumeré. *Nature Nanotechnology.* **2012**, *7, 616-617*.
[2] T. Kothe, S. Pöller, F. Zhao, P. Fortgang, M. Rögner, W. Schuhmann, N. Plumeré *Chem. Eur. J.*, **2014**, *20*, 11029 – 11034.
[3] K. Sokol, D. Mersch, V. Hartmann, J. Z. Zhang, M. M. Nowaczyk, M. Rögner, A. Ruff, W. Schuhmann, N. Plumeré, E. Reisner. *Energy Environ. Sci.*, **2016**, *9*, 3698-3709.
[4] V. Hartmann, T. Kothe, S. Pöller, E. El-Mohsnawy, M. M. Nowaczyk, N. Plumeré, W. Schuhmann, M. Rögner *Phys. Chem. Chem. Phys.*, **2014**, *16*, 11936 - 11941.
[5] M. M. Nowaczyk, N. Plumeré, in *Adv Biochem Eng Biotechnol*, DOI: 10.1007/10_2016_7, Springer, **2016**, "Biophotoelectrochemistry of Photosynthetic Proteins".
[6] M. M. Nowaczyk, N. Plumeré. *Nature Chemical Biology* **2016**, *12*, 990-991.
[7] F. Zhao, S. Hardt, V. Hartmann, H. Zhang, M. M. Nowaczyk, M. Rögner, N. Plumeré, W. Schuhmann, F. Conzuelo. *Nature Communication*, **2018**, 9 (1), 1973

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> IL185. Invited Lecture
Symposium PLANT-7 Electronic Photosynthesis (Eleni Stavrinidou)

BIOELECTRONICS FOR MONITORING AND CONTROLLING PLANT PHYSIOLOGY

Authors: Eleni Stavrinidou[1]
Presenting Author: Eleni Stavrinidou
1) Linköping University

Organic bioelectronics is a field that couples organic electronics with biology. The coupling can be bidirectional for sensing and actuation where a biological process is monitored by an electronic device or an electronic device triggers a biological reaction. Based on polymers, organic bioelectronic devices are ideal for translating addressing electronic signals to complex ionic outputs and vice versa. These devices have been primarily applied to mammalian systems for control of physiology, neural prosthesis, therapy and diagnostics.

Here I will present our efforts towards developing bioelectronic devices for controlling and monitoring plant physiology. By using a bioelectronic electrophoretic device we can deliver phytohormones to plants with high spatiotemporal resolution. In a first example we deliver the hormone auxin in the growth medium of Arabidopsis and demonstrate electronic control of root growth. As a second example we further engineering our devices for in-vivo delivery of the hormone abscisic acid in the leaf apoplast of tobacco plants. We demonstrate the control of stomata and get insight on the ABA signal propagation in the apoplast. In addition, we don't observe a significant wound response from the mechanical insertion of the device. Furthermore, we are developing sensor devices that are based on transistors for monitoring metabolites in plants and demonstrate real-time monitoring of glucose export from isolated chloroplasts. As a next step we are developing devices that will allow in vivo monitoring. Our technology can be used as a research tool from plant biologists and can find possible application in agriculture and in forestry.

> IL186. Invited Lecture
Symposium PLANT-7 Electronic Photosynthesis (Eleni Stavrinidou)

BIO PHOTO VOLTAIC (BPV) - DEVELOPMENT AND POSSIBLE AREAS OF APPLICATION

Authors: Paolo Bombelli[Unive]
Presenting Author: Paolo Bombelli
1) University of Cambridge

Photosynthetic (micro)organisms are capable to generate electrons that can be harvested by a suitable electrochemical setup and be used as electrical current. This concept forms the basis of Bio Photo Voltaic (BPV)[1]. The electrical output obtained from these bio electrochemical systems has improved considerably over the last few years, with the maximum reported being in the region of ca.4A m-2 for the systems operated with cyanobacteria cells [2].

A number of aspects have been considered for enhancing the electrical output and make the BESs suitable for actual applications. These include the availability of electrons from the organisms involved, the transfer of electrons outside the cellular body and interface to

the electrode, and the nature of the materials used to build the electrochemical setup. With the aim to focus on possible areas of application I will present ongoing projects where BPV systems constitute a useful source of electricity in, for example, off-grid locations. I will discuss, for example the use of BPV systems to run environmental sensor for wastewater monitoring in Bangalore India. In addition, I will also promote the idea to use of BPVs as educational toolkit for disseminating knowledge related with energy and sustainability in schools. This was done by promoting the creation of open-source algal-BES prototypes [3].

References

[1] Energy Environ. Sci., 2015, 8: 1092-1109.
[2] Nature Energy, 2018, 3: 75–81.
[3] http://www.iaacblog.com/programs/bio-bottle-voltaic-bbv/

> IL187. Invited Lecture
Symposium PLANT-7 Electronic Photosynthesis (Eleni Stavrinidou)

PHOTOCONVERTERS FROM PHOTOSYNTHETIC BACTERIA

Authors: Gianluca Maria Farinola[1], Roberta Ragni[1], Francesco Milano[2], Gabriella Buscemi[1], Marco Lo Presti[1], Angela Agostiano[1,2], Massimo Trotta[2]
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The photosynthetic bacterial Reaction Centers are trans-membrane photoenzymes able to efficiently convert photons collected by the light harvesting pigments into charge separated states which eventually fuel the biochemical photosynthetic machinery. The unmatched unitary photoconversion efficiency of RC, optimized by evolution, makes this system attractive for integration in biohybrid assemblies for solar energy conversion. The reaction Center (RC) of *Rhodobacter sphaeroides* R26 has been demonstrated to be robust enough to be easily handled, isolated and implemented into electrochemical or optoelectronic devices[1]. Unfortunately, isolated RC suffers from a limited absorption cross-section in the visible spectral region, where the sun reaches the maximum irradiance. We have increased the light harvesting ability of isolated RC by covalently linking designed organic fluorophores acting as artificial antennas to improve the enzyme absorption cross-section[2]. We have also demonstrated the inclusion of oriented RC into soft nanostructures like organic polymersomes and polydopamine nanoparticles with RC, maintaining its electrochemical features in these soft structures[3]. More interestingly, we covalently affixed the RC on hydrogen bonded molecular semiconductor (epindolidione) directly on devices after incubation with suberate as the linker[4]. Oriented assembly of RC units onto the gate electrode of an EGOT device enabled photo gating of the transistor.

In conclusion, our studies disclose new bio-hybrid supramolecular structures for sunlight photoconversion and for light-triggered bioelectronics, by combination of tailored functional molecules with natural photoconverters. In particular, the highly selective covalent functionalization of the bacterial RC of *Rhodobacter sphaeroides* with either tailored molecular fluorophores or molecular semiconductors enables its integration in optoelectronic or photoelectrochemical devices, demonstrating the possibility of producing new generation materials for optoelectronics by biotechnological routes.

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References

1 F. Milano, A. Punzi, R. Ragni, M. Trotta, and G.M. Farinola, Adv. Funct. Mater. 1805521 (2018)
2 F. Milano, R. R. Tangorra, O. Hassan Omar, R. Ragni, A. Operamolla, A. Agostiano, G. M. Farinola and M. Trotta, Angewandte Chemie 124 (44), 11181-11185 (2012)
3 G.M. Farinola, R. Ragni, F. Milano, S. La Gatta, R. R. Tangorra, M. Mastropasqua Talamo, M. Lo Presti, A. Agostiano, S. R. Cicco, A. Operamolla, O. Hassan Omar, M. Trotta, SPIE Proceedings Volume 9944, Organic Sensors and Bioelectronics IX, 994406 (2016)
4 E. D. Głowacki, R. R. Tangorra, H. Coskun, D. Farka, A. Operamolla, Y. Kanbur, F. Milano, L. Giotta, G. M. Farinola and N. S. Sariciftci, J. Mater. Chem. C, 3, 6554-6564 (2015)

> IL188. Invited Lecture
Symposium PLANT-7 Electronic Photosynthesis (Eleni Stavrinidou)

PURPLE BACTERIAL PHOTOSYNTHETIC COMPLEXES FOR PHOTOELECTROCHEMICAL AND SENSORY APPLICATIONS

Authors: Swee Ching Tan[1], Michael R. Jones[2], Sai Kishore Ravi[1]
Presenting Author: Swee Ching Tan
1) Department of Materials Science and Engineering, National University of Singapore 2) School of Biochemistry, University of Bristol

Introduction
Research on solar energy conversion by photosynthetic proteins in a device setting has been primarily directed toward optimising charge separation and mediation, with much less attention paid to developing new device architectures for specialized applications. In this work, three different approaches to constructing bio-hybrid devices are presented that aid in achieving enhanced photocurrent for direct electricity generation and for sensory applications.

Results and Discussion
(I) ***Solid-state Device with Directional Energy Transfer***: A solid-state device architecture enabled by a mechanoresponsive gel electrolyte (Fig 1a) that can be applied under non-denaturing conditions is demonstrated. Devices exhibited enhanced current stability and a maximal photo-response of ≈860 μA cm^{-2}, a 5-fold improvement over the best of previous comparable devices mimicking the modular antenna/transducer architecture (Fig 1b-d).

(II) ***Tandem Cell*****:** Using a tandem configuration, two different variants of optically complementing photoproteins (green and red) were stacked in a device as per band-theory principles (Fig 1e) that showed up to ≈20 % stronger currents than could be obtained with two optically-identical layers or cells in mixed-configuration. In addition, the use of PEDOT:PSS as an electrode material resulted in a 12-fold enhancement in photocurrent density compared to that achievable with platinum (Fig 1 f, g).

(III) ***Photosynthetic e-Skin*** This work presents a proof-of-concept electronic skin (Fig 1h), integrated with pigment-proteins that not only shows an ability to sense low-pressure touch stimuli (down to 3000 Pa, Fig 1i) but also to sense low-intensity UV A or UV B radiation (down to 0.01 mW/cm^2, Fig 1j) and generate electrical power of up to 260 nW/cm^2 in response to white light excitation. The scalability of this biohybrid photosynthetic electronic skin is demonstrated with repeatable cycles of touch response (Fig 1k). Touch recognition and tracking is also demonstrated with multi-pixel sensors.

Methods
All biological materials and device fabrication routes are described in ref [1-3]. Devices were tested under 1 Sun illumination with Keithley 2400 sourcemeter.

Acknowledgments
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References

1. S. K. Ravi, D. J. Swainsbury, V. K. Singh, Y. K. Ngeow, M. R. Jones and S. C. Tan, *Advanced Materials*, 2018, **30**, 1704073.
2. S. K. Ravi, T. Wu, V. S. Udayagiri, X. M. Vu, Y. Wang, M. R. Jones and S. C. Tan, *Advanced Materials*, 2018, **30**, 1802290.
3. S. K. Ravi, Z. Yu, D. J. Swainsbury, J. Ouyang, M. R. Jones and S. C. Tan, *Advanced Energy Materials*, 2017, **7**, 1601821.

> IL189. Invited Lecture
Symposium PLANT-7 Electronic Photosynthesis (Eleni Stavrinidou)

MANIPULATION OF ELECTRON TRANSFER IN BACTERIAL REACTION CENTERS

Authors: JoAnn Williams[1], James Allen[1]
Presenting Author: JoAnn Williams
1) School of Molecular Sciences, Arizona State University

The bacterial reaction center has proven to be a versatile platform for experimentation on light-induced electron transfer pathways, as the characteristics of the cofactors can be modified by replacement or alteration of interactions with nearby amino acid residues. Properties such as the energetics of the bacteriochlorophylls are integral to their electron transfer capabilities. For example, the midpoint potential of the bacteriochlorophyll dimer, P, that serves as the primary electron donor, can be tuned over a range of several hundred mV. As a result, we can explore the factors that enable oxidation of a variety of metal complexes by P^+.

Our previous work has shown that a redox-active Mn cofactor can be incorporated by modifications on the surface of the reaction center. In this case, addition of carboxylate residues creates a mononuclear binding site for Mn near the dimer. For reaction centers having a P/P^+ midpoint potential higher than the Mn(II)/Mn(III) midpoint potential of 625 mV at pH 9, optical measurements show that the bound Mn rapidly reduces P^+ in a first-order reaction. To expand beyond a simple mononuclear Mn cofactor, we are examining two distinct experimental approaches.

One approach is to introduce metal clusters into artificial proteins that interact with the reaction center. For example, four-helix bundles bind dinuclear metal cofactors including Fe and Mn, which can be oxidized by the reaction center. We have modeled these Mn-proteins as docking near the periplasmic surface of the reaction center in a similar position to that of the native secondary donor, cytochrome c_2. Once the Mn-protein is bound, the dinuclear cluster is capable of rapid electron transfer to reduce P^+. The incorporation of artificial proteins as electron donors provides flexibility in cofactor composition and attachment strategies.

Alternatively, synthesized metal clusters as secondary donors offer multinuclear centers with a variety of initial oxidation states. We have investigated electron transfer to reaction centers from Mn-oxides, including Mn_2O_3, $CaMn_2O_4$, Mn_3O_4, and MnO_2, by testing the binding of these Mn-oxides to modified reaction centers. The results show P^+ reduction for each of the Mn-oxides, with the yield of electron transfer generally inversely correlated with the initial Mn oxidation state.

Together, these outcomes expand the tools available for the design of new electron transfer pathways. Manipulation of features such as high oxidation states and the coupling of electron and proton transfer is applicable to understanding water oxidation and engineering novel reactions catalyzed by metal clusters.

> IL191. Invited Lecture
Symposium PLANT-8 Circadian clock in Plants, Algae and Cyanobacteria (David Somers)

A ROLE FOR THE NUCLEAR PORE IN NUCLEOCYTOPLASMIC PARTITIONING AND THE MAINTENANCE OF TEMPERATURE COMPENSATION IN THE PLANT CIRCADIAN CLOCK

Authors: David Somers[Ohio], Yeon Jeong Kim[Ohio], Iris Meier[Ohio], Byungha Lee[Ohio], Anna Dobritsa[Ohio], Hua Shi[Ohio]
Presenting Author: David Somers
1) Ohio State University

Nucleocytoplasmic shuttling is essential for proper clock function although few components of the nuclear pore (NP) have been implicated as regulatory in any circadian system (1, 2). We have identified mutations in NP components in Arabidopsis that lengthen circadian period and are associated with mRNA export defects and misregulated protein sumoylation. NUCLEAR PORE ANCHOR (NUA), with similarity to the inner nuclear basket proteins Tpr (Translocated Promoter Region), Mlp1/Mlp2 (Myosin-like proteins 1 and 2), and Megator is located at the inner nuclear envelope within the “nuclear basket” of the NP (3).

We find circadian period is lengthened in *nua* mutants by 1-2 hours, but the severity of the defect is strongly influenced by ambient temperature, resulting in a marked loss of temperature compensation. *nua* mutants exhibit high accumulation of SUMO conjugates, similar to the effects of mutations in the SUMO protease, ESD4 (3). However, *nua esd4* double mutants show temperature-dependent epistasis, indicating that the role of NUA in the circadian system is likely not due to SUMO-dependent effects on the clock.

Analysis of mRNA and protein levels of known clock genes show only select and limited effects on mRNA and protein levels. Strikingly, double mutants between *nua* and select clock mutants point to TOC1 as a key element affected by NUA loss. The short period *toc1* mutant (21 hrs) is fully epistatic to the long period of the *nua* mutant at all temperatures tested.

Nuclear/cytoplasmic fractionation and confocal microscopy show higher nuclear levels of TOC1 in the *nua* mutant, consistent with the longer period, and the epistatic effect of the *toc1* mutant on the *nua* period phenotype. These and other data will be presented to suggest that one role for NUA in the circadian system is in the regulation of the nucleocytoplasmic partitioning of select clock proteins.

References

1. Jang, A. R., Moravcevic, K., Saez, L., Young, M. W., & Sehgal, A. (2015) *PLoS. Genet.* **11**, e1004974.
2. Macgregor, D. R., Gould, P., Foreman, J., Griffiths, J., Bird, S., Page, R., Stewart, K., Steel, G., Young, J., Paszkiewicz, K. *et al.* (2013) *Plant Cell* **25**, 4391-4404.
3. Xu, X. M., Rose, A., Muthuswamy, S., Jeong, S. Y., Venkatakrishnan, S., Zhao, Q., & Meier, I. (2007) *Plant Cell.* **19(5)**, 1537-1548.

> IL190. Invited Lecture

Symposium PLANT-8 Circadian clock in Plants, Algae and Cyanobacteria (David Somers)

HOW PROTEINS CAN TELL TIME

Authors: Andy LiWang[1]
Presenting Author: Andy LiWang
1) University of California, Merced

Introduction

Circadian clocks arose in organisms as an adaptation to the rotation of the earth. These biochemical chronometers have three components: (***1***) *oscillators* that generate a 24-hr biochemical rhythm; (***2***) *signal transduction pathways* that transmit this rhythm to (***3***) *transcription factors* that then activate genes with a day/night cycle. Thus, circadian clocks produce involuntary anticipation of sunrise and sunset by controlling daily rhythms of gene expression. In this talk, the mechanism of a model system, that of cyanobacteria, will be described. Briefly, this circadian clock depends on phosphorylation, long-range allostery, dynamics, and protein metamorphosis. Because a simple mixture of cyanobacterial clock proteins and ATP generates a persistent macroscopic rhythm, the mechanism of this clock can be studied in real time over several days. Recently, the LiWang lab has reconstructed the clock in vitro to encompass the oscillator, signal transduction pathways, a transcription factor, and DNA promoter.

Methods

The circadian clock system of cyanobacteria was reconstituted in vitro using recombinant proteins. This system includes core oscillator components, signal transduction enzymes, a clock-controlled transcription factor, promoter DNA, and ATP. This mixture generates an autonomous macroscopic circadian rhythm of protein-protein and protein-DNA interactions which are monitored over several days using fluorescence and NMR spectroscopies.

Results & Discussion

As the attached figure shows, the LiWang lab can now observe in vitro and in real-time circadian rhythms of (*1*) oscillator, (*2*) signal transduction, and (*3*) transcription factor components using fluorescence, and DNA using NMR spectroscopy over several days. These rhythms arise from periodic interactions driven by the oscillator that ultimately activate daily transcription factor-DNA binding. It was found that each day the oscillator transiently opens a window through which it transmits biochemical signals downstream.

Conclusions

The ability to reconstitute in vitro the cyanobacterial circadian clock system allows highly precise measurements of every clock component in real time, bringing to light the succession of transient interactions separated in time optimized to regulate gene expression according to time of day.

Acknowledgements

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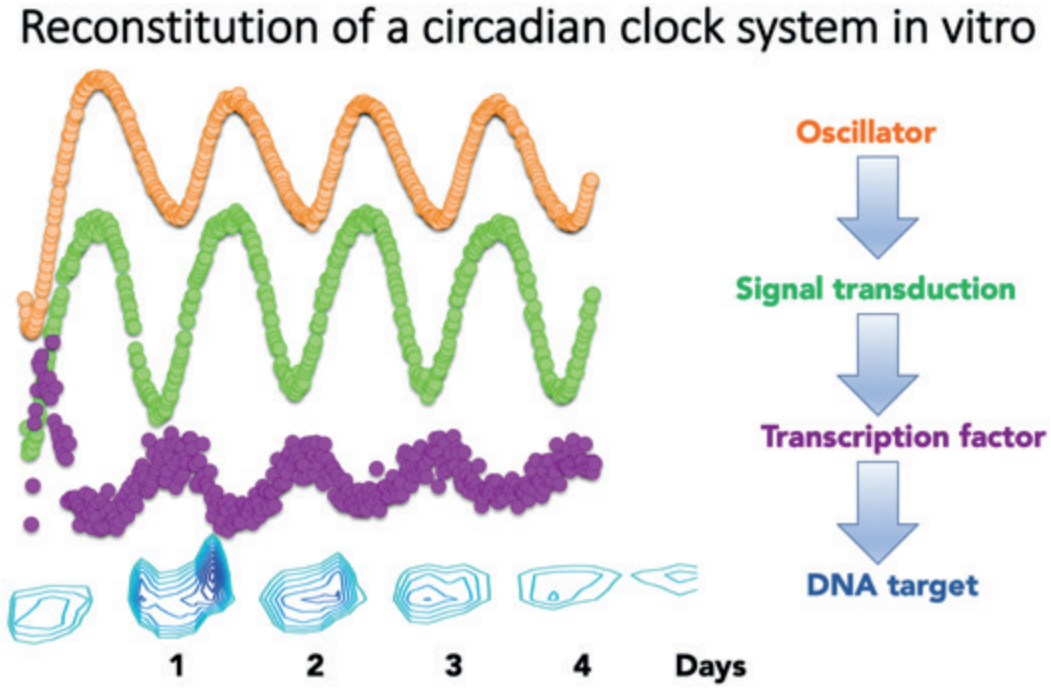
Continued


Reconstitution of a circadian clock system in vitro
Oscillator
Signal transduction
Transcription factor
DNA target
1
2
3
4
Days

> IL194. Invited Lecture

Symposium PLANT-8 Circadian clock in Plants, Algae and Cyanobacteria (David Somers)

PHYTOCHROME INTERACTING FACTOR REGULATION OF PHOTOSYNTHETIC ENTRAINMENT OF THE PLANT CIRCADIAN OSCILLATOR

Authors: Rachel Green[1], Ekaterina Shor[1], Raya Potavskaya[1], Ayelet Kurtz[1], Enamul Huq [2]
Presenting Author: Rachel Green
1) The Hebrew University of Jerusalem 2) The University of Texas at Austin

The plant circadian (~24 hour) system is extremely sensitive to both light quality and quantity. It has long been known that light can entrain the circadian oscillator directly via photoreceptors. However more recently it has also been shown that light indirectly affects the oscillator via photosynthesis and that the relationship between the circadian system and photosynthesis is reciprocal – the circadian system regulates photosynthesis and the products of photosynthesis feedback and control the oscillator. Using a range of techniques, we have shown that members of the bHLH transcription factor PHYTOCHROME INTERACTING FACTOR (PIF) family mediate photosynthate entrainment of the circadian oscillator with sucrose from photosynthesis directly affecting PIF binding to the promoters of key circadian oscillator genes. We have also shown that light quality affects PIF-mediated photosynthetic entrainment, surprisingly with red and blue lights having the opposite effects. In this talk the complex interactions between photosynthetic and photoreceptor entrainment of the oscillator and their possible adaptive significance will be discussed.

> IL193. Invited Lecture
Symposium PLANT-8 Circadian clock in Plants, Algae and Cyanobacteria (David Somers)

DYNAMIC PLASTICITY OF THE ARABIDOPSIS CIRCADIAN OSCILLATOR IN RESPONSE TO SUGAR SIGNALS

Authors: Alex Webb[1]
Presenting Author: Alex Webb
1) University of Cambridge

The defining characteristic of circadian rhythms is that they have a period of about 24 h. However, circadian period is not fixed, it is variable. Many signals regulate the speed of the circadian clock in a reversible manner, with the effect dependent on the time of the day, a process we have called dynamic plasticity (Webb et al., 2019 *Nature Comms* **10**, 550). We have been investigating the mechanism and purpose of the dynamic plasticity of the circadian oscillator to sugar signals. We have previously demonstrated that sugars can speed up the circadian oscillator and identified three signalling pathways by which sugars act, including one dependent on the regulation of the expression of the circadian clock gene *PSEUDO-RESPONSE REGULATOR 7* (*PRR7*) by the energy sensitive transcription factor bZIP63 (Frank et al., 2018 *Current Biol.* **28**, 2597-2609). We are now investigating why the circadian oscillator responds to sugar signals. We will describe new data that demonstrates that the circadian oscillator responds to endogenous changes in sugars that affect the entrainment of the circadian oscillator to light intensity and photoperiod dependent on the correct functioning of *PRR7*. Experimentation and mathematical modelling demonstrate that responses of the circadian oscillator to responses to moderate changes in light intensity can be explained in terms of changes in sugar signalling associated with the management of transient starch reserves in the leaf. Our data suggest that response the circadian oscillator to endogenous sugar signals is required for the correct timing of internal events with respect to the environment.

> IL192. Invited Lecture
Symposium PLANT-8 Circadian clock in Plants, Algae and Cyanobacteria (David Somers)

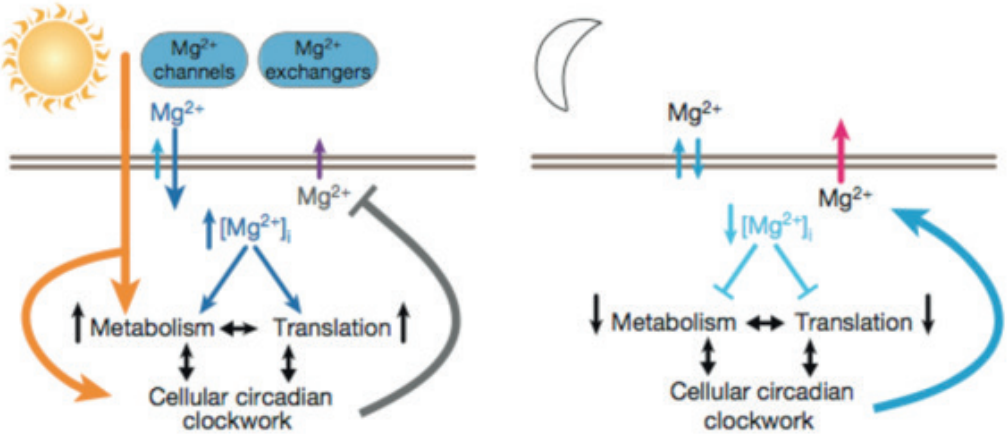
CELLULAR CIRCADIAN TIMEKEEPING AND DYNAMIC REGULATION OF MAGNESIUM

Authors: Gerben van Ooijen[1]
Presenting Author: Gerben van Ooijen
1) University of Edinburgh

Magnesium ions are essential to the activity of >600 enzymes and many other aspects of the biochemistry of all life. It is required for the synthesis of DNA and RNA, and also acts as an indispensable cofactor for ATP as the energy currency of life itself.

We recently discovered circadian rhythms in the concentration of magnesium ions within the cells of humans, algae, and fungi, with an amplitude sufficiently large to affect cellular protein synthesis rates. Evidence is now accumulating for dynamic cell-autonomous regulation of magnesium availability, to fine-tune rates of metabolic and biochemical processes.

Using the unicellular green alga *Ostreococcus tauri*as a model cell of reduced genetic and structural complexity, we aim to advance fundamental understanding of circadian magnesium oscillations and the resultant regulation of cellular biochemistry throughout the 24h cycle. In this talk, I will provide an update on our efforts to a) identify the transmembrane proteins that act as the molecular mediators of dynamic fluxes of magnesium, and b) identify the effects of magnesium flux on overall cellular biochemistry and performance.



> IL195. Invited Lecture

Symposium PLANT-8 Circadian clock in Plants, Algae and Cyanobacteria (David Somers)

ORIGINATION OF THE CIRCADIAN CLOCK SYSTEM IN STEM CELLS REGULATES CELL DIFFERENTIATION IN ARABIDOPSIS THALIANA.

Authors: Kotaro Torii[1,2], Keisuke Inoue[2], Motomu Endo[1]
Presenting Author: Motomu Endo
1) Nara Institute of Science and Technology, Japan 2) Kyoto University, Japan

The circadian clock regulates various physiological responses. To achieve this, both animals and plants have distinct circadian clocks in each tissue that are optimized for that tissue's respective functions. However, if and how the tissue-specific circadian clocks are involved in specification of cell types remains unclear. Here, we developed a new analytics pipeline for single-cell transcriptomes and found that the *Arabidopsis* circadian clock is involved in the process of cell differentiation. Direct repression of *LATE ELONGATED HYPOCOTYL* (*LHY*) expression by BRI1-EMS SUPPRESSOR 1 (BES1) triggers reconstruction of the circadian clock in stem cells. The reconstructed circadian clock regulates cell differentiation through fine-tuning of key factors for epigenetic modification, cell-fate determination, and the cell cycle. Thus, the establishment of circadian systems precedes cell differentiation and specifies cell types.

> IL196. Invited Lecture

PLANT-9 Reactive Oxygen Species in Photosynthetic Organisms: Sensing and Defense (Eva Hideg)

COORDINATION OF CHLOROPLASTIC AND MITOCHONDRIAL ROS SIGNALING

Authors: Jaakko Kangasjärvi[1]
Presenting Author: Jaakko Kangasjärvi
1) University of Helsinki

Plant chloroplasts and mitochondria work together to supply the cell with energy and metabolites. In these organelles, reactive oxygen species (ROS) are formed as by-products of the electron transfer chains Signaling from chloroplasts and mitochondria is partly dependent on ROS, which serve as versatile signaling molecules regulating many aspects of development, stress signaling, systemic responses, and programmed cell death. This communication network affects gene expression in the nucleus where numerous signals are perceived and integrated. However, the molecular mechanisms of the coordinated action of the two energy organelles in response to environmental cues, such as changing light intensity are poorly understood. An Arabidopsis mutant lacking nuclear protein RCD1 has defects both in the mitochondria and in the chloroplasts; it has altered formation of ROS in chloroplasts, and continuously expresses the Mitochondrial Dysfunction Stimulon (MDS) genes. RCD1 is a multidomain protein where its RST domain mediates interaction with transcription factors and the PARP and WWE-domains bind poly(ADP-ribose), a polymer synthetized by PARPs on nuclear acceptor proteins. RCD1 serves as scaffold for nuclear protein complex formation and chloroplastic ROS affect its abundance, redox state and oligomerization. RCD1 interacts with ANAC013 and ANAC017, transcriptional regulators of ROS-related mitochondrial retrograde signaling. Inactivation of *RCD1* increases expression of the MDS genes regulated by ANAC013 and ANAC017, including genes for *AOXs*. Accumulation of AOXs and other MDS gene products in the mitochondria affect respiration and energy metabolism, and alter electron transfer in the chloroplasts, leading to decreased ROS production in the chloroplasts and increased protection of photosynthetic apparatus. RCD1-dependent regulation is also involved in 3\'-phosphoadenosine 5\'-phosphate (PAP)-mediated retrograde signaling; a significant overlap exists between genes negatively regulated by RCD1, the MDS genes, and genes affected by PAP. Sensitivity of RCD1 to organellar ROS provides feedback control of nuclear gene expression and RCD1 integrates retrograde signals from both chloroplasts and mitochondria to exert its influence on nuclear gene expression. This way chloroplasts may affect mitochondria through RCD1. In addition, analysis of photosynthetical functions in the presence of mitochondrial inhibitors showed that MDS genes influence not only the mitochondria, but also the chloroplasts. Overall, RCD1 allows dialog between retrograde signals of both energy organelles. This makes it an important previously unknown regulator of plant energy metabolism.

> IL197. Invited Lecture

PLANT-9 Reactive Oxygen Species in Photosynthetic Organisms: Sensing and Defense (Eva Hideg)

NANOBIOTECHNOLOGY APPROACHES FOR UNDERSTANDING AND ENGINEERING THE ROLE OF PLANT ROS

Authors: Juan Pablo Giraldo[Unive]
Presenting Author: Juan Pablo Giraldo
1) University of California, Riverside

A limitation to advancing our knowledge of how plants respond to and tolerate stress is understanding the role of short-lived and highly reactive plant signaling molecules of oxygen (ROS) both within and between cells. Current approaches to monitor and manipulate ROS are based on biotechnology tools limited to a few plant model systems lacking the temporal resolution to sense or manipulate rapid or long-term changes in ROS in specific subcellular compartments. Herein, we apply nanobiotechnology approaches to study the dual role of ROS as signaling and damaging molecules in plant stress response. Single walled carbon nanotubes (SWCNT) can act as genetic element delivery platforms to plant organelles responsible for ROS generation (e.g. chloroplasts) and as optical nanosensors for monitoring ROS in plants in real-time. SWCNT can act as in vivo ROS sensors that fluoresce in the near infrared where living tissues are relatively transparent. They do not photobleach and have the potential for millisecond temporal resolution and single molecule detection. Cerium oxide nanoparticles (nanoceria) are potent in vivo catalytic ROS scavengers that can be targeted to chloroplasts for specific ROS manipulation in plant subcellular compartments including hydroxyl radicals (˙OH) that lack enzymatic scavenging pathways. Nanoceria delivered to chloroplasts increase plant photosynthetic performance under stresses including high light, heat, chilling and salinity. Compared with plants without nanoparticles, plants embedded with nanoceria exhibit an increase in photosynthetic performance such as quantum yield of photosystem II, carbon assimilation rates, and Rubisco carboxylation rates, and biomass under conditions of abiotic stress. Nanoceria plant ROS scavenging ability also modulates the activities of K+ efflux channels, improving K+ retention in leaf mesophyll cells, a key trait associated with salinity stress tolerance. Catalytic ˙OH scavenging by nanoceria in leaves results in about three-fold lower NaCl-induced mesophyll K+ efflux compared to control leaves upon exposure to salinity stress, indicating a significant improvement in mesophyll K^+ retention. The ROS-activated plasma membrane nonselective cation channels (ROS-NSCC) were identified as the main ˙OH-inducible K^+ efflux channels which are tuned by nanoceria. Nanobiotechnology offers unique high spatial and temporal resolution tools to study the role of ROS as signals encoding and regulating specific plant abiotic stress responses. Synthetic and versatile nanoparticle-based tools have the potential to be more easily translated from plant model systems to diverse plant species for understanding plant physiology.

> IL198. Invited Lecture

PLANT-9 Reactive Oxygen Species in Photosynthetic Organisms: Sensing and Defense (Eva Hideg)

THE ABNORMAL FORMATION OF SHORT-LIVED SINGLET OXYGEN THREATENS PLANTS WITH PROGRAMMED CELL DEATH: STUDIES IN CELL CULTURES AND THE ARABIDOPSIS MUTANTS ABA1 AND MAX4

Authors: Juan B. Arellano[1]
Presenting Author: Juan B. Arellano
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Singlet oxygen ($^1O_2$) is a reactive oxygen species that is formed constitutively in photosystem II (PSII) of plant chloroplasts. Plants can cope with the basal production of $^1O_2$ under normal environmental conditions, but high levels of $^1O_2$ are produced in response to excess excitation energy in PSII. The temporal profile of $^1O_2$ emission endogenously produced by PSII reaction centre in aqueous buffers indicates that attempts to analyse it in chloroplasts are unlikely to be rewarded with success without significant advance in the sensitivity of the detection equipment. Despite its short lifetime, $^1O_2$ is a signalling molecule able to trigger defence responses in plant cells. In Arabidopsis cell suspension cultures (ACSC), high light (HL) stress induces acclimation and the upregulation of transcripts highly correlated with $^1O_2$ formation at early times. When the HL stress ceased, ACSC recovered the initial rate of oxygen evolution and cell growth continued. A high correlation was observed with the transcriptional profiles of two Arabidopsis mutants *aba1* and *max4* with defects in the biosynthesis pathways of two key carotenoid-derived plant hormones. When $^1O_2$ was artificially photosensitized by Rose Bengal (RB), the photosynthetic activity was inhibited and programmed cell death (PCD) was activated. The condensation of the cell protoplast could be observed when light-grown cell wells were subjected to RB. In contrast, when dark-grown cell cultures were subjected to RB under low to medium light conditions, PCD was suppressed, indicating that the $^1O_2$-mediated signalling pathway in ACSC requires functional chloroplasts. Analysis of up-regulated transcripts in light-grown ACSC, treated with RB in the light, showed that both $^1O_2$-responsive transcripts and transcripts with a key role in PCD like ethylene and jasmonic acid were present. Thylakoids of *aba1*produced twice as much $^1O_2$ as thylakoids of *max4* and wild type plants when illuminated with HL. A loss of connectivity between PSII units was rationalized as the main cause for the high yield of $^1O_2$ production in *aba1*. Chloroplast aggregation followed by chloroplast rupture and eventual cell death was observed by confocal imaging of the fluorescence emission of leaf cells of *aba1*. In contrast, *max4* did not show evidence of $^1O_2$-mediated cell death. In conclusion, ACSC and *aba1* may serve as alternative models to other $^1O_2$ overproducing mutants of Arabidopsis for investigating $^1O_2$-mediated cell death.

Acknowledgements
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References

Sánchez-Corrionero et al. (2017). J. Plant Physiol. 216:188-196.
Gutiérrez et al., (2014). Journal of Experimental Botany. 65: 3081-3095.
Li et al., (2012). Photosynthesis Research. 112: 75-79.
Gutiérrez et al., (2011). Plant Signaling and Behavior. 6:1937-1942.
González-Pérez et al., (2011). Plant Physiology. 156:1439-1456.

> IL199. Invited Lecture

PLANT-9 Reactive Oxygen Species in Photosynthetic Organisms: Sensing and Defense (Eva Hideg)

PRODUCTION OF REACTIVE OXYGEN SPECIES DURING LEAF SENESCENCE

Authors: Anja Krieger-Liszkay[1], Karin Krupinska[2], Ginga Shimakawa[1]
Presenting Author: Anja Krieger-Liszkay
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Generation of reactive oxygen species (ROS) in chloroplasts may play a crucial role in triggering the initiation of leaf senescence[1]. We studied the generation of ROS and changes in the photosynthetic electron transport chain in two barley varieties. During senescence chlorophyll content decreased and photosynthetic electron transport was inhibited as shown for flag leaves collected from barley varieties Lomerit and Carina grown in the field and in controlled conditions. Spin trapping electron paramagnetic resonance (EPR) was used to investigate the production of reactive oxygen species in thylakoid membranes during senescence[2]. EPR measurements were performed with specific spin traps to discriminate between singlet oxygen on one hand and reactive oxygen intermediates on the other hand. The results show that the generation of reactive oxygen intermediates increases in both varieties during senescence. Singlet oxygen increased only in the variety cv. Lomerit while it remained constant at a low level in the variety cv. Carina. In field grown material, photosystem II activity decayed much earlier in Lomerit than in Carina, while no difference was observed in material grown under controlled conditions. Measurements in the presence of inhibitors of photosystem II and of the cytochrome b6f complex revealed that in senescing leaves reduction of oxygen at the acceptor side of photosystem I was the major, but not the only source of superoxide anions. This study shows that during senescence the production of individual reactive oxygen species varies in different barley varieties and different growth conditions. Abiotic stresses like UV, fluctuating light, extreme temperatures or temporary drought may affect photosystem II activity and singlet oxygen production in field-grown Lomerit, thereby inducing a different senescence scenario than under controlled conditions where only superoxide is generated in both, Lomerit and Carina.

References

[1] Krieger-Liszkay A, Krupinska K, Shimakawa G (2019) The impact of photosynthesis on initiation of leaf senescence. Physiol Plant. doi: 10.1111/ppl.12921.
[2] Krieger-Liszkay A, Trösch M, Krupinska K. (2015) Generation of reactive oxygen species in thylakoids from senescing flag leaves of the barley varieties Lomerit and Carina. Planta 241:1497-508.

> IL200. Invited Lecture

PLANT-9 Reactive Oxygen Species in Photosynthetic Organisms: Sensing and Defense (Eva Hideg)

TAKING SIDES IN THE BATTLE OF PRO- AND ANTIOXIDANTS: DIVERSE ROLES OF PHENOLIC COMPOUNDS IN STRESSED PLANTS

Authors: Éva Hideg[1], Gyula Czégény[1], Kristóf Csepregi[1], Arnold Rácz[1]
Presenting Author: Éva Hideg
1) Department of Plant Biology, University of Pécs, Hungary

Plants synthesize a wide range of phenolic secondary metabolites, and many of these accumulate in response to stress conditions [1]. Many flavonoids and phenolic acids have high reactivity to ROS [2], implying that these compounds may have a direct antioxidant function in plant tissues, too. These ROS neutralizing reactions, however, may yield radical products, and prooxidant activities of dietary flavonoids have been in the focus of medicinal biochemistry research for decades [3]. In addition to direct reactions with ROS, plant specific class III peroxidases (POD) also oxidize phenolic compounds as substrates [4] and the aim of this study was to explore whether phenolic derived radicals are hazardous components of the plants' defence system.

Growth chamber experiments with tobacco (*Nicotiana tabacum* L.) plants exposed to near ambient supplemental UV doses over a sub-ambient PAR background showed that the photochemical acclimation of leaves was achieved by adjusting the ratio of regulated and non-regulated photochemical quenching. Enzymatic antioxidant defence was stronger in UV-B acclimated leaves and it was focussed on hydrogen peroxide neutralization [5], which is explained by the potential UV-B photo-cleavage of hydrogen peroxide [6]. A selective enhancement of POD isoforms [7] and a phenolic substrate preference of these enzymes [8] suggest a distinctness of the antioxidant response to UV. The main phenolic compounds of tobacco leaves were quercetin, quercetin-rutinoside, chlorogenic acid, and caffeic acid. Only some of these substrates were restored by ascorbate when oxidised by POD, and a detectable radical product was not a common characteristic either.

Although other plant species featuring different phenolic profiles may produce oxidized products which were not included in this model study, our results suggests that the pro-oxidant function of phenolic compounds imposes a relatively small risk *in planta*.

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References

[1] Dixon RA, Paiava NL (1995) Plant Cell 7, 1085-1097.
[2] Csepregi K, Hideg É (2018) Phytochem Anal 29, 129-136.
[3] Cao G, Sofic E, Prior, RL (1997) Free Rad Biol Med 22, 749-760.
[4] Hiraga S, Sasaki K, Ito H, Ohashi Y, Matsui H (2001) Plant Cell Physiol 42, 462-468.
[5] Czégény Gy, Mátai A, Hideg É (2016) Plant Sci 248, 57-63.
[6] Czégény Gy, Wu M, Dér A, Eriksson LA, Strid Å, Hideg É (2014) FEBS Lett, 588, 2255-2261.
[7] Rácz A, Hideg É, Czégény Gy (2018) J Plant Physiol, 221, 101-106.
[8] Rácz A, Czégény Gy, Hideg É (2019) see congress abstract in this issue.

> IL201. Invited Lecture
PLANT-9 Reactive Oxygen Species in Photosynthetic Organisms: Sensing and Defense (Eva Hideg)

THE USE OF GENETICALLY-ENCODED PEROXIDE SENSORS TO INVESTIGATE LIGHT RESPONSES IN ARABIDOPSIS

Authors: Dominique Arnaud[1], Deirdre McLachlan[2], Michael Deeks[1], Alistair Hetherington[2], Nicholas Smirnoff[1]
Presenting Author: Nicholas Smirnoff
1) University of Exeter, UK 2) University of Bristol, UK

Hydrogen peroxide (H_2O_2) is formed during metabolism, usually *via* dismutation of superoxide, whose production is mediated by oxidases and electron transport processes. In plants, photosynthesis is a major source of H_2O_2 *via* oxygen photoreduction at PSI in chloroplasts (Mehler reaction) and by the glycolate oxidase reaction in peroxisomes (photorespiration). H_2O_2 and other reactive oxygen species (ROS) are potentially damaging and their concentrations are kept low by the antioxidant system. However, they can act as signalling molecules. Investigating the role of H_2O_2 has been problematic because commonly-used measurement techniques lack spatial and chemical specificity. Genetically-encoded fluorescent sensors with high specificity for peroxide can overcome some of these problems. We have previously used HyPer, a YFP-based probe. HyPer targeted to chloroplasts and nuclei allowed visualisation by confocal microscopy of photosynthetically produced H_2O_2 in chloroplasts and photosynthesis-dependent appearance of H_2O_2 in the nucleus. These experiments provided evidence that H_2O_2 produced by chloroplasts can move to the nucleus where it influences gene expression. HyPer expression is subject to silencing and, critically, its fluorescence is pH sensitive. To avoid these problems, we have used an alternative sensor, roGFP2-Orp1. It contains a yeast thiol peroxidase (Orp1) fused to a redox-sensitive GFP (roGFP2). Oxidation of an Orp1 cysteine by H_2O_2 initiates a redox relay resulting in roGFP2 oxidation, measured by a change in the fluorescence excitation spectrum. We have produced transgenic Arabidopsis with the probe targeted to cytosol, nucleus, chloroplast stroma, peroxisomes, mitochondria and apoplast. Confocal microscopy confirmed that roGFP2-Orp1 was accumulated in the expected subcellular compartments. Light causes probe oxidation in a dose-dependent manner in the chloroplast stroma while nuclear localised roGFP-Orp1 oxidises only in high light. Although peroxisomal roGFP-Orp1 is more oxidised than stromal roGFP2-Orp1 in low light, it is not more oxidised at high light intensity. The results suggest that catalase-based H_2O_2 removal in peroxisomes has a high capacity while chloroplasts release H_2O_2 in high light. In plants, ascorbate has a role in H_2O_2 removal through ascorbate peroxidase (APX) activity. Arabidopsis expressing cytosolic/nuclear roGFP2-Orp1 was crossed with the ascorbate deficient mutant *vtc2-4*. *vtc2-4* has decreased activity of the biosynthetic enzyme GDP-L-galactose phosphorylase and contains ~20% of wild type ascorbate. As predicted, roGFP2-Orp1 is more oxidised in the *vtc2-4* mutant background under “basal” conditions and shows more oxidation than wild type following H_2O_2 addition and high light exposure. The results show that roGFP2-Orp1 will be a valuable tool in probing the dynamics and function of photosynthetically-produced H_2O_2 and the functions of various components of the antioxidant system.

> OC090. Oral Communication

PLANT-9 Reactive Oxygen Species in Photosynthetic Organisms: Sensing and Defense (Eva Hideg)

AN ANIONIC PORPHYRIN: TPPS, A GOOD CANDIDATE FOR APDT IN AGRONOMY

Authors: Veronica Ambrosini[labor], Abdel-Kayyoum Madi[labor], Mohammad Issawi[labor], Vincent Sol[labor], Catherine Riou[labor]
Presenting Author: Veronica Ambrosini
1) Laboratory PEIRENE, Faculty of Science and Techniques, University of Limoges

Recently, antimicrobial photodynamic treatment (APDT) in agronomy, relying on the use of photosensitizers, was sought to fight against crop pathogens such as bacteria and fungi without disturbing plant growth and development. When subjected to UV-visible light, photosensitizers, such as porphyrins, generate reactive oxygen species (ROS) that induce cell death. In previous studies, we showed that the anionic porphyrin, even tested at high concentration, did not alter tomato and Arabidopsis plant growth making them good candidates for further use in APDT [1-3] . Thus, the next step to validate our approach was to test anionic porphyrin on grapevine, the most important crop in our region and one of its pathogens Botrytis cinerea both separately and afterwards together. In this presentation, we will show our initial results on grapevine and fungus. When tested at 12.5 µM TPPS did not disturb grapevine growth in vitro. We showed that plantlets were able to resist by producing large amounts of antioxidants, such as thiols, ascorbate and tocopherols. By contrast, under light treatment, Botrytis mycelium growth was definitively hampered at 1.5 µM TPPS that corresponds to the minimum fungicidal concentration. This result was encouraging due to low TPPS concentration being able to kill the fungus without disturbing grapevine growth. We also checked TPPS effect on conidia germination relevant leaf infection. Biochemical and cellular investigations were performed for both Botrytis mycelium and spore germination in liquid medium, and in contact with leaves to defend our strategy. In this work we present all the data that gives us the confidence in developing APDT in agronomy; in the context of conventional pesticide reduction, for the improvement of healthy environmental practices.

References

[1] Issawi, M., Sol, V., Riou, C. (2018) Plant photodynamic stress: What\'s New? Front. Plant Sci., 9: 681- 690.
[2] Guillaumot, D., Issawi, M., Da Silva, A., Leroy-Lhez, S., Sol, V., Riou, C. (2016) Synergistic enhancement of tolerance mechanisms in response to photoactivation of cationic tetra (N-methylpyridyl) porphyrins in tomato plantlets. J. Photochem. Photobiol. B: Biology, 156: 69–78.
[3] Issawi, M., Guillaumot, D., Sol, V., Riou, C. (2018) Responses of an adventitious fast-growing plant to photodynamic stress: comparative study of anionic and cationic porphyrin effect on Arabidopsis thaliana. Physiol. Plant. 162: 379–390.

> OC091. Oral Communication

PLANT-9 Reactive Oxygen Species in Photosynthetic Organisms: Sensing and Defense (Eva Hideg)

PHOTO-ACTIVATED DEFENSE STRATEGIES IN MUSHROOMS – AN OVERLOOKED SOURCE FOR NEW PHOTOPHARMACEUTICALS?

Authors: Bianka Siewert[1], Fabian Hammerle[1], Isabella Bingger[2], Ursula Peintner[3], Jean-Luc Wolfender[4], Andrea Pannwitz[5], Sylvestre Bonnet[5], Hermann Stuppner[1]
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1) Institute of Pharmacy/Pharmacognosy, Center for Molecular Biosciences Innsbruck (CMBI), Center for Chemistry and Biomedicine, University of Innsbruck 2) Department of Biotechnology, Management Center Innsbruck 3) Institute of Microbiology, University of Innsbruck 4) School of Pharmaceutical Sciences, EPGL, University of Geneva 5) Leiden Institute of Chemistry, Leiden University

The splendid colors of mushrooms (Basidiomycetes) are based on a vast array of different pigments. While plenty of these colorants are chemically elucidated, their ecological function has yet to be fully uncovered (Spiteller, 2008). Based on the structural similarity to well-known photosensitizers (e.g. bisanthrones, anthraquinones, and harmanes) we hypothesized that fungi produce them as part of a subtle photo-activated defense mechanism. Moreover, we believe that these photosensitizers can be utilized as photopharmaceuticals.

Methods

Extracts of dried fruiting bodies of several European dermocyboid Cortinarii were prepared and submitted to a photo-activity screening workflow (Siewert et al., 2019). In detail, the chemical profile of light-absorbing metabolites was analyzed, the ability to produce singlet oxygen was tested, and the photocytotoxicity was evaluated. Furthermore, to test the hypothesis, molecular network analysis and dereplication studies were performed with selected species of the genus Cortinarius.

Results & Discussion

Based on the photo-activity workflow, the most prominent dermocyboid species was selected and its photosensitizers isolated by applying a bioactivity-guided workflow. Chemical analysis disclosed that the most prominent photosensitizer is a biphyscion. While it is non-toxic in the dark, it showed an EC_{50} of 0.7 µM under blue light irradiation (468 nm, 9.3 J/cm²) against cells of a lung cancer cell-line (A549). The molecular network analysis of the several Cortinarius species provided further evidence that next to the isolated biphyscion several other promising photosensitizers exist in this genus.

Conclusion

Starting with a hypothesis based on the structural similarity between well-established photosensitizers and the coloring principles of fruiting bodies we were able to assign a new putative ecological role to select fungal pigments. Furthermore, we were able to show that these entities are promising candidates for new photopharmaceuticals.

Acknowledgmen

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References

Siewert, B, Vrabl, P, Hammerle, F, Bingger, I, and Stuppner, H (2019). A convenient workflow to spot photosensitizers revealed photo-activity in basidiomycetes. *RSC Adv.* 9(8), 4545-4552. doi: 10.1039/c8ra10181g.

Spiteller, P (2008). Chemical Defence Strategies of Higher Fungi. *Chemistry – A European Journal* 14(30), 9100-9110. doi: 10.1002/chem.200800292.

> OC092. Oral Communication

PLANT-9 Reactive Oxygen Species in Photosynthetic Organisms: Sensing and Defense (Eva Hideg)

ANTIOXIDANT AND MORE - THE ROLE OF VITAMIN B6 IN PLANT UV-ACCLIMATION

Authors: Gyula Czégény[1], László Kőrösi[2], Åke Strid[3], Éva Hideg[1]
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Solar ultraviolet radiation (UV, 290-400 nm) is an important regulator of plant growth and development [1]. The metabolism of reactive oxygen species (ROS) is a general inherent of this regulation and thus it is well-balanced by the antioxidant systems. The synergistic effect of UV and other abiotic factors may change this sensitive balance, and lead to oxidative damage [2].

Previously, we showed that plant UV-responses at antioxidant level are focussed on effective hydrogen peroxide (H_2O_2) scavenging [3]. The role of H_2O_2 is crucial in UV-exposed leaves, since in addition to increasing cellular H_2O_2 concentrations, UV-B is also capable of photoconverting H_2O_2 to highly oxidizing hydroxyl radicals [4].

Vitamin B_6 and its vitamer derivatives are members of several biosynthetic pathways and contribute to various stress response pathways in plants [5]. Vitamin B_6 production is regulated by the downstream signaling of the UVR8 photoreceptor, and it is enhanced by the UV [6]. In this study, we examined the influence of vitamin B_6 on the enzymatic antioxidant defence *in planta*. *Arabidopsis thaliana* plants (C24 wild type and *rsr4-1* mutant affected in its vitamin B_6 content [7]) were exposed to supplemental UV in growth chambers for 4 days. Our results emphasise the importance of efficient H_2O_2 scavenging under UV pressure and suggested a possible indirect role of vitamin B_6 in that. Furthermore, we also demonstrated direct antioxidant capacities of B_6 vitamers against the four major ROS *in vitro* [8].

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References

[1] Jansen, M.A.K., Gaba, V., Greenberg, B.M. (1998) Trends Plant Sci. 3: 131–135.
[2] Hideg, É., Jansen, M.A.K., Strid, Å. (2013) Trends Plant Sci. 18: 107-115.
[3] Czégény, Gy., Mátai, A., Hideg, É. (2016) Plant Sci. 248: 57-63.
[4] Czégény, Gy., Wu, M., Dér, A., Eriksson, L.A., Strid, Å., Hideg, É. (2014) FEBS Lett. 588: 2255-2261.
[5] Chen, H., Xiong, L. (2005) Plant J. 44: 396–408.
[6] Ristilä, M., Strid, H., Eriksson, L. A., Strid, Å., Sävenstrand, H. (2011) Plant Physiol. and Biochem. 49: 284–292.
[7] Wagner, S., Bernhardt, A., Leuendorf, J.E., Drewke, C., Lytovchenko, A., Mujahed, N., Gurgui, C., Frommer, W.B., Leistner, E., Fernie, A.R., Hellmann, H. (2006) Plant Cell 18: 1722-1735.
[8] Czégény, Gy., Kőrösi L., Strid, Å., Hideg É. (2019) Sci. Rep. 9: 1259.

> IL207. Invited Lecture

Symposium PLANT-10 Photoreceptors and Plant Development (Carlos Ballaré)

MOLECULAR BASIS OF PLANT RESPONSES TO UV-B

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Presenting Author: Gareth Jenkins
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UV-B wavelengths initiate a range of regulatory responses in plants that modify morphology, metabolism and physiology, and include changes in biochemical composition that promote UV-protection and defence against pests and pathogens. Photomorphogenic responses to UV-B are mediated by the photoreceptor UV RESISTANCE LOCUS8 (UVR8). UVR8 signaling leads to the regulation of transcription of numerous genes that underpin photomorphogenic responses.

UVR8 is a 7-bladed b-propeller protein that exists as a homodimer in the absence of UV-B. UV-B photoreception causes rapid dissociation of the dimer into monomers to initiate signaling and hence gene expression through interaction with CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) and a number of transcription factors. Interaction with REPRESSOR OF UV-B PHOTOMORPHOGENESIS (RUP) proteins promotes reversion of monomer to dimer.

Under continuous photoperiodic illumination with white light containing a low fluence rate of UV-B a dimer/monomer photoequilibrium is established where approximately 30% of the protein is present as the monomer. UV-B-acclimated plants respond to increased UV-B exposure to initiate gene expression, but the response can occur without a change in the proportion of monomer. In these plants there is an increased interaction of monomer with both COP1 and RUP2. A model is presented to explain how UV-B-acclimated plants respond to elevated levels of UV-B.

> IL202. Invited Lecture
Symposium PLANT-10 Photoreceptors and Plant Development (Carlos Ballaré)

BLINDED BY THE LIGHT - CHLOROPLAST RETROGRADE SIGNALS SUPPRESS SEEDLING PHOTOMORPHOGENESIS

Authors: Charlotte Gommers[1,2], Elena Monte[2]
Presenting Author: Charlotte Gommers
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Plants obtain and process information about their light environment to optimally use the available light for growth. Seedlings in the dark undergo skotomorphogenic development, to facilitate emergence from the soil. The first exposure to light induces photomorphogenesis: growth arrest of the hypocotyl, unfolding of the apical hook and opening, expansion and greening of cotyledons. If during this process, e.g. by excessive light, chloroplast development is inhibited, retrograde signals are released towards the nucleus and suppress photomorphogenic development.

This chloroplast-mediated suppression of photomorphogenesis is photoreceptor-independent and acts via several different pathways. The chloroplast localized protein GENOMES UNCOUPLED1 (GUN1) acts as a central regulator and is needed to suppress the transcription factors GOLDEN2-LIKE1 (GLK1) and GLK2, which target genes for chloroplast recovery and cotyledon opening. We now show that nuclear gene repression, induced by GUN1-mediated retrograde signals acts via increased activity of a plant-specific class of histone de-acetylases. These transcriptional repressors are specifically induced in cotyledons when chloroplast development is chemically inhibited and mediate repression of photomorphogenesis-promoting genes. We additionally show that chloroplast retrograde signals induce ethylene response factors, which results in the inhibition of cotyledon opening in the light, without changes in ethylene synthesis.

Our data point towards different pathways that all contribute to the suppression of seedling photomorphogenesis when chloroplast biogenesis is disrupted in sub-optimal environments. We combine eco-physiology, retrograde signaling and epigenetics to elucidate the role of chloroplast during environmental signaling.

> IL204. Invited Lecture
Symposium PLANT-10 Photoreceptors and Plant Development (Carlos Ballaré)

PHYTOCHROME SIGNALING AND THE CONTROL OF PHOTOMORPHOGENESIS

Authors: Meng Chen[1]
Presenting Author: Meng Chen
1) University of California, Riverside

Phytochromes are red and far-red photoreceptors that regulate every facet of plant development and growth. When seedlings emerge from the soil and encounter light for the first time, phytochromes trigger a developmental transition from a dark-grown program called skotomorphogenesis to a light-dependent program called photomorphogenesis. The photomorphogenetic program enables the biogenesis of photosynthetically-active chloroplasts and thus transitions seedlings into a photoautotrophic lifestyle. Chloroplast biogenesis requires the activation of photosynthesis-associated genes encoded by both the nuclear and plastidial genomes. It is well understood that light triggers the translocation of phytochromes from the cytoplasm to the nucleus to activate photosynthesis-associated nuclear-encoded genes, but how phytochromes – which do not localize in plastids – control the expression of photosynthesis-associated plastid-encoded genes (*PhAPG*s) remains elusive. The plastidial genome is transcribed by two types of RNA polymerase: a phage-type nuclear-encoded RNA polymerase that transcribes housekeeping genes and a bacterial-type plastid-encoded RNA polymerase (PEP) that transcribes *PhAPG*s. Our genetic studies on phytochrome signaling have serendipitously revealed that phytochrome signaling and the PEP are connected by a dual-targeted nuclear/plastidial protein named HEMERA (HMR). While nuclear HMR is a transcriptional activator required for phytochrome signaling, plastidial HMR is a PEP-associated protein essential for *PhAPG* expression. In my talk, I will discuss our latest work on the mechanistic link between phytochrome signaling and the regulation of plastidial transcription.

> IL203. Invited Lecture
Symposium PLANT-10 Photoreceptors and Plant Development (Carlos Ballaré)

FAR-RED INDUCED SHOOT-ROOT SIGNALING BY A MOBILE TRANSCRIPTION FACTOR HY5

Authors: Kasper van Gelderen[1], Valérie Hoogers[1], Chiakai Kang[1], Ronald Pierik[1]
Presenting Author: Kasper van Gelderen
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Plants can see competitors through the reflection of Far-Red light from plant leaves using the phytochrome photoreceptors. Plant vegetation is Far-Red-enriched due to reflection and transmission of Far-Red by leaves. This Far-Red light can be used by plants to avoid competitors by growing away from this neighbor shade and helps them to maximize light capture for photosynthesis. Far-Red detection also causes plants to reduce their root growth, possibly to conserve resources. This 'shade avoidance' is evolutionary useful, but the growth investment reduces crop yield in high-density monocultures. Plant roots do not directly perceive the Far-Red light signal and we discovered the mechanism through which this light signal travels towards the root. We show that a mobile transcription factor protein, HY5, is able to travel through the vasculature from shoot to root, where it affects the growth of lateral roots by suppressing the signaling and transport of the plant hormone auxin, a classical regulator of lateral root development. Grafting experiments confirmed previous results that HY5 can move from shoot to root and we are looking at the specific tissues HY5 is being transported to and the roles it might play in these tissues. Long distance signaling is a phenomenon which occurs often in plant responses to light and I will share our current research on how nutrient signaling, hormones and transcription factors integrate these long-distance signaling events into growth adaptation.

> IL205. Invited Lecture
Symposium PLANT-10 Photoreceptors and Plant Development (Carlos Ballaré)

THE CONTROL OF FLOWERING TRANSITION IN THE PRESENCE OF COMPETING NEIGHBORS

Authors: Vinicius Costa Galvão[1], Christian Fankhauser[1]
Presenting Author: Vinicius Costa Galvão
1) University of Lausanne, Center for Integrative Genomics

Plants depend on sunlight to fuel photosynthesis. Therefore, growing with potentially reduced light availability, as encountered in dense plant communities, constitutes a threat for plant growth and development. Plants perceive potential competitors because of the reflected far-red (FR) light from neighbors, resulting in reduced red (R)/FR ratio (low R/FR), which leads to the conversion of phytochromes (phy) photoreceptors to their inactive Pr form. In the shade-intolerant plant *Arabidopsis thaliana* neighbor detection triggers organ elongation to outgrow competitors and precocious flowering. Phytochromes are major regulators of shade-induced flowering, however, the molecular mechanism underlying this response is poorly understood.

In Arabidopsis, the mechanism linking low R/FR ratio perception leads to enhanced *FT* (and *TSF*) expression in the vasculature in a photoperiod-dependent manner, in agreement with the attenuated low R/FR response of the photoperiodic mutant *constans* (*co*). We show that a subset of the bHLH transcription factors PHYTOCHROME-INTERACTING FACTORS (PIF) function genetically downstream of phytochromes to regulate flowering time through *FT* and *TSF* expression in response to low R/FR. In agreement, *FT* and *TSF* increased expression under low R/FR is attenuated in loss-of-function mutants. Moreover, we provide evidences that PIF proteins directly regulate TSF expression by directly binding to PBE-box located at its promoter region.

> IL206. Invited Lecture
Symposium PLANT-10 Photoreceptors and Plant Development (Carlos Ballaré)

SOIL SALINITY INHIBITS PLANT SHADE-AVOIDANCE

Authors: Scott Hayes[1], Chrysoula K. Pantazopoulou[2], Kasper van Gelderen[2], Emilie Reinen[2], Adrian L. Tween[2], Ashutosh Sharma[3], Micheal de Vries[4], Salomé Prat[1], Robert C. Schuurink[4], Christa Testerink[5], Ronald Pierik[2]
Presenting Author: Scott Hayes
1) CNB-CSIC 2) Utrecht University 3) The University of Bristol 4) Amsterdam University 5) Wageningen University and Research

Global food production is set to keep increasing despite a predicted decrease in total arable land. To achieve higher production, denser planting will be required on increasingly degraded soils. When grown in dense stands, crops elongate and raise their leaves in an effort to reach sunlight, a process termed shade-avoidance. Shade is perceived by a reduction in the ratio of red (R) to (FR) light and results in the stabilisation of a class of transcription factors known as PHYTOCHROME INTERACTING FACTORs (PIFs). PIFs promote the accumulation of auxin and enhance auxin sensitivity, which promotes cell wall loosening and drives elongation growth. Despite our molecular understanding of shade-induced growth, little is known about how this developmental programme is integrated with other environmental factors. Here we demonstrate that low levels of NaCl in soil strongly impair the ability of plants to respond to shade. This block is dependent upon abscisic acid (ABA) signalling and the canonical ABA signalling pathway. Low R:FR light enhances brassinosteroid (BR) signalling through *BRASSINOSTEROID SIGNALLING KINASE 5* (*BSK5*), and leads to the activation of BRI1 EMS SUPPRESSOR 1 (BES1). ABA inhibits *BSK5* up-regulation and interferes with GSK3-like kinase inactivation by the BR pathway, thus leading to a suppression of BES1:PIF function. By demonstrating a link between light, ABA and BR-signalling pathways this study provides an important step forward in our understanding of how multiple environmental cues are integrated into plant development.

> IL208. Invited Lecture
Symposium PLANT-10 Photoreceptors and Plant Development (Carlos Ballaré)

A LIGHT-DEPENDENT MOLECULAR LINK BETWEEN GROWTH AND DEFENSE RESPONSES IN ARABIDOPSIS

Authors: Carlos Ballaré[1, 2,]
Presenting Author: Carlos Ballaré
1) Universidad de Buenos Aires 2) Universidad Nacional de San Martin 3) CONICET, Argentina

Plants detect and respond to the proximity of competitors using light signals perceived by photoreceptor proteins. A low ratio of red to far-red radiation (R:FR ratio) is a signal of competition that is sensed by phytochrome B (phyB). Low R:FR ratios increase the synthesis of growth-related hormones, including auxin and gibberellins. phyB is also an important modulator of hormonal pathways that regulate plant immunity against herbivores and pathogens, including the jasmonic-acid (JA) signaling pathway. Low R:FR ratios down-regulate JA-induced responses. This down-regulation helps the plant to optimize its developmental configuration and resource allocation patterns under conditions of intense competition. In this presentation, I will discuss recent advances in the understanding of the mechanisms that link phyB with JA metabolism, and explore their functional implications. Unveiling the molecular links between photoreceptors and the regulators of plant immunity is important to understand how plants deal with resource allocation tradeoffs under natural conditions.

> P105. Poster

Symposium PLANT-10 Photoreceptors and Plant Development (Carlos Ballaré)

INVESTIGATION OF THE ROLE OF THE TRANSCRIPTIONAL REGULATOR TZP IN BLUE-LIGHT MEDIATED HYPOCOTYL ELONGATION IN ARABIDOPSIS THALIANA

Authors: Mhairi L.H. Davidson[1], Eirini Kaiserli[1]
Presenting Author: Mhairi L.H. Davidson
1) Institute of Molecular, Cell and Systems Biology, University of Glasgow

Introduction

Light is essential for plant growth and development. Tandem-Zinc-finger-Plus3 (TZP) is a transcriptional regulator that plays a major role in integrating light, hormone and clock signalling networks to promote plant growth in response to endogenous and environmental stimuli. Photomorphogenesis is a major transition early in plant development when hypocotyl (embryonic stem) elongation is inhibited, cotyledons (embryonic leaves) open, and greening occurs. TZP is a positive regulator of blue-light-mediated hypocotyl elongation and regulates expression of growth promoting genes (Loudet *et al*., 2008; Perrella *et al*., 2018). In addition, TZP localises to the nucleus in dynamic, transcriptionally active nuclear bodies (Kaiserli *et al*., 2015).

Methods

To further understand how TZP acts as a transcriptional regulator, structure-function analysis is essential. TZP contains a unique C-terminal structure with tandem zinc-fingers (ZF) directly adjacent to a Plus3 domain. Both domain types can interact with nucleic acids and other proteins. Deletion analysis of TZP *in vivo* and phenotypic assays were used to investigate the role of these domains in hypocotyl elongation.

Results and Discussion

Confocal imaging has shown that neither ZF nor Plus3 is required for the nuclear localisation of TZP. Furthermore, Plus3 alone and ZF-Plus3 lose nuclear specificity and can also be observed in the cytosol. Preliminary data suggests that TZP ZF-Plus3 is sufficient for TZP-mediated hypocotyl elongation in response to low blue light. The next step is to assess if the ZF-Plus3 domains of TZP are sufficient to associate with and regulate the expression of TZP target genes.

Conclusions

This project aims to investigate the molecular mechanism of TZP transcriptional regulation and to further understanding of the interactions among the transcriptional regulator TZP, photoreceptors and other light signalling components.

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Conflicts of Interest

None

References

Kaiserli, E., Páldi, K., O'Donnell, L., Batalov, O., Pedmale, U.V., Nusinow, D.A., Kay, S.A., and Chory, J. (2015). Integration of Light and Photoperiodic Signaling in Transcriptional Nuclear Foci. Dev. Cell *35*, 311–321.

Loudet, O., Michael, T.P., Burger, B.T., Le Metté, C., Mockler, T.C., Weigel, D., and Chory, J. (2008). A zinc knuckle protein that negatively controls morning-specific growth in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. *105*, 17193–17198.

Perrella, G., Davidson, M.L.H., O'Donnell, L., Nastase, A.-M., Herzyk, P., Breton, G., Pruneda-Paz, J.L., Kay, S.A., Chory, J., and Kaiserli, E. (2018). ZINC-FINGER interactions mediate transcriptional regulation of hypocotyl growth in Arabidopsis. Proc. Natl. Acad. Sci. 201718099.

> P106. Poster

Symposium PLANT-10 Photoreceptors and Plant Development (Carlos Ballaré)

STUDY OF PHOTORECEPTOR FUNCTION IN CHLAMYDOMONAS REINHARDTII VIA GENOME EDITING

Authors: Olga Baidukova[1], Simon Kelterborn[1], Irina Sizova[1], Francisca Böhning[1], Peter Hegemann[1]
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The complex photoreceptor apparatus of *Chlamydomonas reinhardtii* regulates their life cycle, photosynthesis, and phototaxis. Channelrhodopsins (ChRs), light-gated ion channels that function as sensory photoreceptors, play a crucial role in these behavioural responses. Despite the detailed *in vitro* characterization of the ChRs, there is still not much known about their function *in vivo*.

Since gene editing protocols for this algae became available, we began to shed light on questions related to the photoreceptor function. We used the method of homologous recombination with a CRISPR/Cas9 toolbox for the reliable modification of the photoreceptor genes. Thus two ChRs genes were inactivated in order to differ between their function. According to the phototaxis studies via the light scattering method, ChR2 is the main photoreceptor responsible for phototaxis in the wild type strain CC125, whereas ChR1 is the dominant photoreceptor in most other strains as for example CC 3403, CC 495, cw2 and cw302. Further, to investigate the change in the kinetics of the phototactic response we introduced single amino-acid substitutions such as E123T (ChR2) and E162T (ChR1) and obtained the strains with a faster photocycle. In addition, we focused on the mutations E90 of ChR2 which result in different ion selectivities. Thus the mutation E90Q shows strongly reduced proton selectivity, while E90R results in a Cl^--conducting channel. All the strains are being analyzed by phototaxis assays and single-cell tracking.

> P107. Poster

Symposium PLANT-10 Photoreceptors and Plant Development (Carlos Ballaré)

OSMOTIC STRESS ACTIVATED KINASES IN THE REGULATION OF LIGHT INDUCED CHLOROPLAST MOVEMENTS.

Authors: Olga Sztatelman[1], Ewa Sitkiewicz[1], Justyna Łabuz[2], Grażyna Dobrowolska[1]
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Chloroplast rearrangements triggered by light are a mechanism to optimize light utilization by plants. Chloroplasts in weak light gather at the cell walls perpendicular to light direction, in order to maximize light absorption. In strong light they move to the cell walls parallel to light direction to avoid stress caused by excess light. Changes in phosphorylation are key for the movement mechanism, as protein phosphatase inhibitors block this response. Several proteins involved in the movements have been identified to date. One of the potential regulators of those proteins are SNF1-related kinases 2 (SnRK2s).

SnRK2s are plant specific group of protein kinases regulating responses to adverse environmental conditions, such as salt or drought, and ABA-dependent development. They can be divided in three groups, based on phylogenetic relations and activating factors. Almost all SnRK2s are activated by osmotic stress but those belonging to group III are also strongly activated by ABA. Group II kinases are only weakly activated by ABA, whereas group I kinases are not ABA responsive. The activation of SnRK2s is rapid and leads to phosphorylation of downstream targets, such as dehydrins, transcription factors and RNA binding proteins [1, 2], leading to changes in plant metabolism and acclimation. One of the group of proteins differentially phosphorylated in *snrk2* mutants are proteins involved in light induced chloroplast movement [1].

In order to assess the link between osmotic stress activated kinases and chloroplast movements, we examined the phosphorylation of some of known proteins involved in the movements by SnRK2s. All tested proteins were phosphorylated by several SnRK2 kinases *in vitro*. The Bimolecular Fluorescence Complementation assay showed that selected proteins interact with kinases *in planta* indicating that indeed they could be *bona fide* SnRK2s' substrates. Using mass spectrometry we mapped phosphorylation sites for a selected substrate and confirmed the results by directed mutagenesis. The identified sites were compared with those regulated by light.

SnRK2s may act as negative regulators of light induced chloroplast movements in stress conditions, consistent with observed decrease in chloroplast movements capacity upon salt treatment. Alternatively, they can be involved in light signaling. The second hypothesis is supported by light-dependent increase in the basal activity of two group I kinases, SnRK2.4 and SnRK2.10, in adult leaves and the phenotypes of selected *snrk2* mutants and its regulators.

Acknowledgements

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References

[1] Wang P. et al. (2013) Quantitative phosphoproteomics identifies SnRK2 protein kinase substrates and reveals the effectors of abscisic acid action. PNAS 110(27):11205–10

[2] Maszkowska J. et al. (2019) Phosphoproteomic analysis reveals that dehydrins ERD10 and ERD14 are phosphorylated by SNF1-related protein kinase 2.10 in response to osmotic stress. *Plant Cell Environ* 42(3): 931-946.

> OC093. Oral Communication

Symposium PLANT-11 Short Communications on Plant Photobiology (Massimo Trotta)

MOLECULAR EVOLUTION OF THE ORANGE CAROTENOID PROTEIN: INTER-DOMAIN INTERACTION AND THE ROLE OF THE LINKER IN CAROTENOID TRANSLOCATION

Authors: Fernando Muzzopappa[1], Adjele Wilson[1], Diana Kirilovsky[1]
Presenting Author: Fernando Muzzopappa
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The cyanobacterial Orange Carotenoid Protein (OCP) is a photoactive protein that plays a major role in dissipating the excess energy arriving at the photosynthetic apparatus. The OCP is composed by two domains connected by a flexible loop. The three paralogous of the OCP (OCP1, OCP2 and OCPX) were originated by gene fusion of ancestral domain genes. We report here the first characterization of an OCPX. Using phylogenetic and biochemical approaches, we characterized one OCP from each subfamily focusing on the inter-domain interaction and the role of the linker. Specific amino acids in the linker provided additional regulation by allowing protein deactivation, enhancing antenna binding and regulating the photoactivation. Most of these features are kept by the relatively ancestral OCPX, including the dimer-to-monomer transition already described in OCP1. Our results suggested, that OCP2 had accumulated mutations in specific residues that increased the inter-domain interaction and preserve the fast deactivation. On the other hand, oligomeric regulation was lost in OCP2. During evolution OCP1 deactivation became slower allowing further regulation by interaction with the FRP, a protein which accelerate the deactivation. Both OCP1 and OCPX have conserved the negative regulation of the photoactivation provided by the linker that is important for these OCP which are constitutively expressed. By contrast, OCP2 developed a positive regulation of the photoactivation by the linker, which counteract the strong domain affinity. This allow the OCP2 to be effective in stress conditions.

> OC094. Oral Communication

Symposium PLANT-11 Short Communications on Plant Photobiology (Massimo Trotta)

STRUCTURAL, SPECTROSCOPIC AND FUNCTIONAL CHARACTERIZATION OF HCPs

Authors: Maria Agustina Domínguez Martín[1], Tomas Polivka[4], Markus Sutter[1,2], Bryan Ferlez[1], Sigal Lechno-Yossef[1], Beronda Montgomery[1,3], Cheryl Kerfeld[1,2,3]
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Introduction

In the majority of cyanobacteria, the Orange Carotenoid Protein (OCP) is a photoreceptor responsible for the thermal dissipation of excess light energy captured by the phycobilisome (PBS). It plays a secondary protective effect by disarming reactive oxygen species. Recently, new families of homologs of the constituent domains of the OCP have been identified (Melnicki et al. 2016). Nine different clades of N-terminal domain homologs have been identified across diverse cyanobacteria; these paralogs are named Helical Carotenoid Proteins (HCPs). Each is predicted to bind a single carotenoid. Homologs to the C-terminal domain (CTDHs) have also been found in nearly every genome encoding an HCP. Most likely, OCP was derived from an HCP combined with a CTDH into a single polypeptide. Our investigation focuses on the structure, and function of the HCPs, particularly weather they constitute a modular photoprotective system.

Methods

We overexpressed HCPs in *Tolypothrix* PCC 7601. After purifying the protein by Ni-NTA affinity chromatography followed by size exclusion chromatography, a biochemical and structural characterization, including protein crystallization, was done. The analysis of the carotenoid content was performed by mass spectrometry. The functional characterization included analysis of the oxygen singlet quenching by EPR and phycobilisome quenching assays using fluorescence.

Results and Discussion

We overexpressed and characterized purified HCP2 protein. We report the 1.7 Å crystal structure of HCP2, one of the most widespread HCPs found in nature from the chromatically acclimating cyanobacterium *Tolypothrix* PCC 7601 (Dominguez-Martin et al., submitted). By purifying HCP2 from the native source we were able to identify its natively-bound carotenoid, which is exclusively canthaxanthin. In solution, HCP2 is a monomer with an absorbance maximum of 530 nm. However, the HCP2 crystals have a maximum absorbance at 548 nm, which is accounted by the stacking of the β1 rings of the carotenoid in the two molecules in the asymmetric unit. Regarding function, HCP2 does not quench PBS but it is a good singlet oxygen quencher.

Conclusions

Our results demonstrate how HCPs provide a valuable system to study carotenoid-protein interactions and their spectroscopic implications, and contribute to efforts to understand the functional roles of this large, newly discovered family of pigment proteins, which to-date remain enigmatic.

Acknowledgment

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References

Melnicki M et al. (2016) Structure, Diversity, and Evolution of a New Family of Soluble Carotenoid-Binding Proteins in Cyanobacteria. Molecular Plant

Dominguez-Martin MA et al. (2019) Structural and spectroscopic characterization of HCP2. Submitted to BBA-Bioenergetics

> OC095. Oral Communication

Symposium PLANT-11 Short Communications on Plant Photobiology (Massimo Trotta)

REVEALING THE ULTRAFAST DYNAMICS OF ORANGE CAROTENOID PROTEIN FROM SYNECHOCYSTIS USING TIME-RESOLVED ABSORPTION SPECTROSCOPY

Authors: Stanisław Niziński[1,2], Elena Andreeva[3], Jacques-Philippe Colletier[3], Adjele Wilson[4], Diana Kirilovsky[4], Gotard Burdzinski[1], Michel Sliwa[2]
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Orange Carotenoid Protein (OCP) is a water-soluble pigment protein responsible for dissipation of excited state energy harvested by cyanobacterial antenna complexes, phycobilisomes. OCP performs its photoprotective function in a selective way: in the dark it occurs in inactive form (OCP^O), but after illumination with a blue-green light it undergoes photoconversion with a low quantum yield (< 1%) to quenching-capable form called OCP red (OCP^R). OCP^R interacts with the phycobilisome, causes a decrease of the phycobilisome fluorescence emission intensity and prevents the excitation energy reaching the reaction centers (Kirilovsky, D. & Kerfeld, C. A. Nat. Plants, 2016). The exact mechanism that controls the photoconversion of OCP^O to OCP^R is still not fully understood, and it is essential to obtain a complete picture of lightcyanobacteria interaction. A detailed photoconversion mechanism was recently proposed for echinenone OCP from *Synechocystis* produced in *E. coli* based on fs-ms visible transient absorption (TA) spectroscopy (400-750 nm, Konold *et al*., 2018, JACS): upon absorption of a blue-green photon (475 nm), the carotenoid undergoes $S_0 \rightarrow S_2$ transition, then it decays within hundreds of fs to following excited states, a ps-lived S_1 state coupled to an intramolecular charge transfer ICT state (95%) and a distorted S* state (5%). The S* state is leading (1.5%) to the ground state intermediate, which relaxes to the final OCP^R (< 0.6%) after few milliseconds with the existence of four steps. Formation of S* state is thus important and determines the overall low photoconversion yield, however its dynamics are not clear because it is hindered in the UV-Vis region by S_2 and S_1/ICT absorption bands.

To distinguish different excited states and clarify the deactivation of OCP^O we employed femtosecond transient absorption spectroscopy in the NIR region (750-1400 nm). We observed a short living species (less than 0.2 ps) absorbing at 1050 nm, which is presumably S_2 state. After its decay, a stimulated emission around 1200 nm is growing and disappearing within 1 ps. The fused analysis of NIR and visible region with two different excitations (470 and 540 nm) allows us to separate the contribution of S_1, ICT and S* states and obtain their spectra and dynamics (formation and decay). We found that quantum yield and dynamics in the ground state strongly depend on method used to produce the OCP. Based on these results, we can build an improved scheme of early OCP photoconversion dynamics.

> OC096. Oral Communication
Symposium PLANT-11 Short Communications on Plant Photobiology (Massimo Trotta)

DIFFERENT ROLES FOR ApcD AND ApcF IN SYNECHOCOCCUS ELONGATUS AND SYNECHOCYSTIS SP. PCC 6803 PHYCOBILISOMES

Authors: Pablo Ignacio Calzadilla[1], Fernando Muzzopappa[1], Pierre Sétif[1], Diana Kirilovsky[1]
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The phycobilisome, the cyanobacterial light harvesting complex, is a huge phycobiliprotein containing extramembrane complex, formed by a core from which rods radiate. The phycobilisome has evolved to efficiently absorb sun energy and transfer it to the photosystems via the last energy acceptors of the phycobilisome, ApcD and ApcE. ApcF also affects energy transfer by interacting with ApcE. In this work we studied the role of ApcD and ApcF in energy transfer and state transitions in *Synechococcus elongatus* and *Synechocystis* PCC6803. Our results demonstrate that these proteins have different roles in both processes in the two strains. The lack of ApcD and ApcF inhibits state transitions in *Synechocystis* but not in *S. elongatus*. In addition, lack of ApcF decreases energy transfer to both photosystems only in *Synechocystis*, while the lack of ApcD alters energy transfer to photosystem I only in *S. elongatus*. Thus, conclusions based on results obtained in one cyanobacterial strain cannot be systematically transferred to other strains and the putative role(s) of phycobilisomes in state transitions need to be reconsidered.

> OC097. Oral Communication

Symposium PLANT-11 Short Communications on Plant Photobiology (Massimo Trotta)

FAST PHOTOPROTECTION (qE) IN PLANTS LACKING MINOR LIGHT HARVESTING COMPLEXES AND PHOTOSYSTEM REACTION CORES

Authors: Francesco Saccon[Queen], Vasco Giovagnetti, Alexander Ruban
Presenting Author: Francesco Saccon
1) School of Biological and Chemical Sciences, Queen Mary University of London

Photosynthesis in nature is fuelled by solar light energy. Fast and reversible non-photochemical quenching (qE) is a physiological process that protects the photosynthetic apparatus of plants from harmful unused energy that is accumulated in excess under high light. Despite several decades of qE research, a consensus regarding the site and mechanism of this process is yet to be reached. Here, we use *Arabidopsis thaliana* plants which lack the minor light-harvesting complexes of photosystem II and possess strongly reduced amounts of photosystem reaction cores to investigate the minimum requirements for qE. The thylakoid membranes of these plants contain almost exclusively major light-harvesting complexes II (LHCII). Despite the reduced protein composition of their thylakoids, these plants are still able to form quickly reversible non-photochemical quenching, dependent on trans-thylakoid ΔpH, at similar extents to wild-type plants. Moreover, the qE induced shows the same characteristics as in wild-type plants: (1) the absence of the PsbS protein largely impairs it under physiological ΔpH levels; (2) accumulation of the carotenoid zeaxanthin modulates qE kinetics by accelerating its formation and slowing down its relaxation; (3) low-temperature fluorescence measurements point towards a mechanism of photoprotection that involves aggregation of LHCII. These findings highlight the minimum requirements for qE in plants: ΔpH, LHCII trimers and PsbS.

> OC098. Oral Communication
Symposium PLANT-11 Short Communications on Plant Photobiology (Massimo Trotta)

SILICIFIED STRUCTURES AFFECT LEAF OPTICAL PROPERTIES OF DESCHAMPSIA CESPITOSA FROM DIFFERENT HABITATS

Authors: Mateja Grašič[1], Tamara Saković[1], Katarina Vogel-Mikuš[1,2], Alenka Gaberščik[1]
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Silicon (Si) is an important biomineral in grasses, as it affects plant water and energy balance, and improves plant resistance to pathogens and herbivores. Si mainly accumulates in the epidermis and thus significantly affects leaf optical properties. *Deschampsia cespitosa* L. is a perennial grass that is found in many grassland types, but favourises poorly-drained soils. The objectives of this study were to examine various leaf traits in *D. cespitosa* from different habitats, and the contents of Si in leaves and the corresponding soils. Plants were sampled from four different locations bearing four different habitats. The first two habitats, floodplain of the river Rak near Rakov Škocjan, and intermittent Lake Cerknica, are characterised as wetland sites on carbonate rocks. In contrast, two sites with prevailing calc-alkaline volcanic rocks were chosen, namely a heath under the top of the mountain Komen, and a forest edge at the foothills of the same mountain. Ten plant and soil samples were collected from each location. Leaf morphological and biochemical traits were analysed along with their reflectance and transmittance. Element analysis of leaves and soils was performed using X-ray fluorescence spectrometry. Plant available ($CaCl_2$-extractable) Si levels in the soil were also determined. The soil properties differed significantly between the four habitats, including soil element composition. Contrasting habitat characteristics resulted in differences in leaf morphological, biochemical, and optical properties of samples from the different habitats, along with their leaf element contents. No correlations between total soil Si, plant-available Si, and leaf Si were obtained. However, Si availability affected the properties of Si structures at the leaf surface. Redundancy analysis revealed that among biochemical traits, chlorophyll *a*, chlorophyll *b*, and carotenoids explained 53% of the reflectance spectra variability, while among morphological traits, upper leaf surface prickle hair density and upper cuticle thickness explained 31% of the reflectance spectra variability. Prickle hairs were significantly positively related to leaf reflectance in short wavelengths from UV-B to blue, while upper cuticle thickness was negatively related to leaf reflectance from UV-B to violet. However, in explaining leaf transmittance, only long prickle hair density and short prickle hair length revealed to be significant. These results point out the importance of Si structures at the leaf surface for leaf optical properties of *Deschampsia cespitosa*.

SYMPOSIUM COMMUNICATIONS

ENVIRONMENTAL PHOTOBIOLOGY

> IL210. Invited Lecture
Symposium ENV-1 UV Monitoring for human health (Mario Blumthaler)

UV MONITORING FOR HUMAN HEALTH

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Presenting Author: Mario Blumthaler
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Overexposure to solar UV radiation is a risk for public health. Therefore, it is important to provide information to the public about the level of solar UV. The UV-Index (UVI) is the relevant quantity, expressing the erythemally weighted irradiance to a horizontal plane on a simple scale [WHO]. As solar UV irradiance is strongly variable in time and space, measurements within a network provide the best source of information, provided they can be made available rapidly. However, to ensure the information is reliable, strict QA/QC procedures for the monitoring networks are necessary. In a recent survey, 160 monitoring sites in 25 European countries are described in terms of instrumentation, QA/QC, and publication of data to the Internet [Schmalwieser et al., 2017].

Near real time presentation of the measured UVI on web-pages is the best way to inform the public. Many measurement sites present the UVI as graphs, showing the diurnal variation. In the Austrian UV monitoring network (www.ui-index.at) additionally a regional generalization of the UVI is presented [Schallhart et al., 2008], based on the actual measurements at each site, on a clear sky model calculation for each pixel and on the attenuation by clouds as observed by MSG [Verdebout, 2000]. Every 15 min, a new image from the satellite is received and an updated map is determined. It is intended to expand this map to whole of Europe, enabling near real-time information about the actual UVI at each place in Europe.

The interpretation of the published data of the UVI in terms of the individual exposure dose is heavily impacted by skin type, behaviour, and clothing, and must be learned for each person through experience and guidance. A meaningful suggestion for quantifying the exposure dose is to introduce the 'UV Index hour' as a simple quantity, which can be understood intuitively [Saxebøl, 2000]. From the definition of the UVI, it follows that 1 UVIh corresponds to 90 Jm^{-2} of erythemally weighted exposure. This is close to the so-called 'standard erythema dose SED' (100 Jm^{-2}), which is frequently used for UV exposure by artificial sources.

Generally, reliable knowledge of the actual level of the intensity of erythemally weighted irradiance and its variability forms the basis of education and public awareness.

References

Blumthaler, M. UV monitoring for public health. *Int. J. Environ. Res. Public Health* **2018**, 15, 1723-1733.

Saxebøl, G. UVH—A proposal for a practical unit for biological effective dose for ultraviolet radiation exposure. *Radiat. Prot. Dosim.* **2000**, *88*, 261–261.

Schallhart, B.; Blumthaler, M.; Schreder, J.; Verdebout, J. A method to generate near real time UV-Index maps of Austria. *Atmos. Chem. Phys.* **2008**, *8*, 7483–7491

Schmalwieser, A.; Gröbner, J.; Blumthaler, M.; Klotz, B.; De Backer, H.; Bolsée, D.; Jepsen, N. UV Index monitoring in Europe. Photochem. Photobiol. Sci. **2017**, 16, 1349–1370.

Verdebout, J. A method to generate surface UV radiation maps over Europe using GOME, Meteosat, and ancillary geophysical data. *J. Geophys. Res.* **2000**, *105*, 5049–5058.

WHO; WMO; UNEP; ICNIRP. Global Solar UV Index - A Practical Guide; WHO: Geneva, Switzerland, 2002.

> IL211. Invited Lecture
Symposium ENV-1 UV Monitoring for human health (Mario Blumthaler)

SOLAR UV RADIATION METROLOGY: SUPPORTING HEALTH AND CLIMATE RESEARCH

Authors: Julian Gröbner[1], Gregor Hülsen[1], Luca Egli[1]
Presenting Author: Julian Gröbner
1) PMOD / WRC

Solar UV radiation has important effects on the Atmosphere, Plants and Animals and on human health in particular. While small amounts of solar UV radiation have beneficial effects on human health through the production of Vitamin D, exposure to high doses of solar UV radiation may result in acute and chronic health effects on skin, eye and immune system. While solar radiation measurements have been performed for more than 100 years, measurements of the ultraviolet part of the solar spectrum have shown significant challenges to achieve the desired uncertainties. Only in recent years have methods and procedures been developed to a level were measurements of solar UV radiation have become fully traceable to SI with uncertainties close to what is achievable in the laboratory. The World Calibration Center for UV (WCC-UV) of the World Meteorological Organisation is hosted at PMOD/WRC. Its objective is to harmonise solar UV measurements made by the world-wide community through instrument calibrations and on-site quality assurance. PMOD/WRC is signatory of the CIPM MRA and designated institute for solar irradiance by the Swiss Metrology Institute METAS. The WCCUV has implemented a quality system according to ISO 17025 and has listed 6 Calibration and Measurement capabilities (CMC) in the KCDB of the BIPM, thereby providing solar UV measurements (spectral and broadband) traceable to the SI. The WCCUV operates the QASUME portable reference spectroradiometer for the quality assurance of spectral solar UV irradiance measurements at monitoring sites and provides calibrations of broadband solar UV filter radiometers through its facilities at PMOD/WRC.

> IL214. Invited Lecture
Symposium ENV-1 UV Monitoring for human health (Mario Blumthaler)

UV MONITORING AT HIGH LATITUDES: LINKING ARCTIC AND ANTARCTIC UV MEASUREMENTS

Authors: Kaisa Lakkala[1], Jukka Kujanpää[1], Margit Aun[1,2], Germar Bernhard[3], Outi Meinander[1], Ricardo Sanchez[4], Antti Arola[1], Anu Heikkilä[1], Iolanda Ialongo[1], Niilo Kalakoski[1], Tomi Karppinen[1], Juha M. Karhu[1], Gerrit de Leeuw[1], Alberto Redondas[5], Johanna Tamminen[1]
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1) Finnish Meteorological Institute 2) University of Tartu, Estonia 3) Biospherical Instruments, Inc., San Diego, U.S.A. 4) Servicio Meteorológico Nacional, Argentina 5) Spanish Agencia Estatal de Meteorología, Tenerife

Introduction
The Finnish Meteorological Institute (FMI) started spectral UV measurements at Sodankylä, 67°N, Finland, in 1990. The location of the station at high latitudes gives extra challenges: harsh weather conditions like temperatures falling under -30°C, snow, high cloudiness and high solar zenith angles. Proper quality assurance is essential for performing high quality UV measurements. In this presentation we show quality assurance procedures of FMI's UV measurements and how they are implemented in both Sodankylä and Marambio, 64°S, Antarctica. In collaboration with Servicio Meteorológico Nacional new measurements with GUV multifilter radiometer (GUV) were set up at Marambio in 2017. They continue the measurements of the Antarctic NILU-UV network, which stopped in 2013. FMI is also responsible for several satellite UV products, and validation of the products is shown especially for high latitudes.

Methods
At Sodankylä, two Brewer spectroradiometers and two NILU-UV radiometers are used to monitor UV radiation (litdb.fmi.fi). The quality assurance of spectral UV measurements includes daily housekeeping, regular calibrations, solar comparisons, corrections for cosine error, wavelength shift, temperature dependence, dead time and dark counts. At Marambio, two GUV rotate so that the GUV is replaced each year by a calibrated one. In addition, the GUV measurements are regularly compared with spectral measurements at Sodankylä. The EUMETSAT Satellite Application Facility on Atmospheric Composition Monitoring (AC SAF) UV Data Record product was compared with ground based measurements.

Results and Discussion
Marambio\'s measurements showed that the UV index can be 12 during low total ozone episodes in late spring, while in Sodankylä UV indices in the spring don\'t exceed those measured in the summer. For the period of 1990–2018, a maximum UV index of 6 was measured in 2011 and 2013 at Sodankylä. The validation of the AC SAF UV product showed that for UV doses, the median of relative differences from ground based measurements was less or equal to 10% at 23 sites. There still exist challenges in discrimination of snow and clouds for extreme conditions, like in the spring at some high latitude sites.

Conclusions
Proper quality assurance procedures are crucial for high quality UV measurements at high latitudes. Solar comparisons make possible to quantitatively compare UV measurements performed at different locations. Satellite measurements are essential to complement the observations from the sparse ground-based UV network as they provide global spatial coverage. However, the inhomogeneous surface and low solar angles increase the uncertainty of satellite UV retrievals at high latitudes, and ground measurements are crucially needed for the continuous validation of satellite UV products and development of satellite UV processors.

Acknowledgements
We thank the operators of the measurement sites in Sodankylä and Marambio. The AC SAF team is acknowledged.

> IL213. Invited Lecture
Symposium ENV-1 UV Monitoring for human health (Mario Blumthaler)

EXPANSION OF THE GERMAN SOLAR UV MONITORING NETWORK

Authors: Sebastian Lorenz[1], Cornelia Baldermann[1], Daniela Weiskopf[1]
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1) Federal Office for Radiation Protection, Germany

Introduction

The German Federal Office for Radiation Protection operates a nationwide network for solar ultraviolet (UV) radiation monitoring in cooperation with the Federal Environment Agency, Germany\'s National Meteorological Service and other associated institutions. The network includes twelve stations at defined locations in Germany, at which the solar UV irradiance is spectrally resolved measured from sunrise to sunset.

The collected data are used to
• determine current values of the irradiance weighted with the standard erythemal action spectrum and the UV Index,
• derive recommendations for protection of the public, and
• get valuable knowledge on short- and long-term trends of ground level UV irradiance.

The expansion of the German solar UV monitoring network with a special focus on the applied devices, validation and measurement results as well as the communication of the current UV exposure to the public will be presented.

Background and main elements of the network expansion

Until 2017, scanning double monochromators (DM) were used within the network for spectrally resolved measurements to meet our requirements on accuracy. However, these devices are expensive and maintenance-intensive. In addition, the measurement time of several minutes is a drawback in case of fast changing cloud conditions. An alternative system was found with a diode array radiometer using BTS technology (BTS). Comparative validation measurements of BTS and DM systems show that the more cost effective BTS systems achieve sufficient stray light reduction (dynamic range) with a shorter measurement time than DM, and high spectral resolution. Thus, the spectral solar UV irradiance can now be determined more precisely at fast changing cloud conditions. Therefore, three BTS diode array radiometer were acquired. One was installed in the high mountain region Alps where often fast changing cloud conditions as well as the highest solar UV irradiance in Germany occur.

To inform the public about the current UV exposure the BfS publishes daily courses of the UV Index as derived from the measurements of all measurement stations continuously updated over the day. However, the information is generally valid to the region of the measurement station due to the strong dependence of the solar UV irradiance on the cloudy conditions. For comprehensive information, the number of measurement stations of the German solar UV monitoring network is insufficient. To this end, the existing network will be expanded to twenty additional stations equipped with small and low-cost UV Index sensors (filter/broadband radiometer) which achieve the desired level of accuracy for UV Index determining. The *expansion* is made cost-effective due to a cooperation with the German ODL (ambient gamma dose rate) network with its 1800 stations.

Conflicts of Interest

There are no conflicts of interest.

Continued


Bundesamt für Strahlenschutz

> IL212. Invited Lecture
Symposium ENV-1 UV Monitoring for human health (Mario Blumthaler)

PERSONAL UV EXPOSURE FROM AMBIENT UV RADIATION

Authors: Alois W. Schmalwieser[1]
Presenting Author: Alois W. Schmalwieser
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People are exposed to solar radiation throughout their whole life. Exposure to UV radiation is vital but also holds serious risks. The quantification of human UV exposure is a complex issue. UV exposure is directly related to incoming UV radiation as well as to a variety of factors such as the orientation of the exposed anatomical site in respect to the sun and the duration of exposure. This includes behaviour and clothing.

The use of badge sensors allows assessing the UV exposure of differently oriented body sites. Such UV devices have been available for over 40 years and a variety of measuring campaigns have been undertaken since then. A short overview will be given on what knowledge is available.

Another possibility to asses UV exposure of different body sites is the application 3d-body models. These allow calculating the UV exposure over the whole body. Precise model calculations need ambient UV measurements as input parameters or as calibration factor, because atmospheric input parameters are not always available with satisfactory accuracy. A few examples of state of the art models will be presented.

Measured or modelled UV Exposure can be expressed as “Exposure Ratio To Ambient” (ERTA). The ERTA expresses the percentage of irradiance received by a certain body site compared to the irradiance received by a horizontally oriented receiver (free horizon). The ERTA depends on the orientation of body site in respect to the sun and on the local environ. The orientation changes with solar elevation and with the posture. With that, the ERTA (when given as a function of solar elevation, body site and activity) can be used in conjunction with ambient UV radiation to calculate the personal UV exposure of people.

A rarely investigated, but a very important topic is clothing of people. Clothes generally absorb UV radiation, but release certain parts of the skin to the sun. Therefore, clothing habits must be known when estimating UV exposure of body sites. A few examples for introducing clothing habits in personal UV exposure estimations will be given.

This talk will give an overview of the state of the art in estimating personal UV exposure from ambient UV radiation.

> IL209. Invited Lecture
Symposium ENV-1 UV Monitoring for human health (Mario Blumthaler)

IS EXPOSURE TO UV RADIATION A VIABLE CHOICE FOR VITAMIN D PRODUCTION IN NORTHERN EUROPE OF TODAY?

Authors: Ann Webb[1], Richard Kift[1], Mark Farrar[1], Lesley Rhodes[1]
Presenting Author: Ann Webb
1) University of Manchester

The measurement of UV radiation is often, at least partially, justified on the grounds of public health issues. Traditionally this was sunburn and skin cancer (risk), more recently vitamin D synthesis (benefit) has also gained support. Ability to balance UV benefit and risk depends on location (climate), skin type and behaviour, the latter determined by culture, employment and personal choice. Alternatively, vitamin D can be acquired by ingestion though modern diets are generally low in vitamin D and regular supplementation would be the most reliable way to achieve this.

Previous work has shown how the risk/benefit balance for UV exposure can be achieved in the UK (and similar) climate[1,2]. That is, vitamin D needs can be met without risk of sunburn. Although exposure times are short, at least for a white-skinned population, there is a requirement to expose sufficient unprotected skin in the warmest months of the year (more than simply hands and face). Thus, while our solution to acquiring vitamin D needs through sun exposure sounds simple we ask whether it is pragmatic with modern lifestyles, working practices and multicultural population.

Analysis of sun exposure diaries recording time outdoors and clothing worn, from a range of studies, provides us with a picture of how close different sections of the population come to meeting our exposure guidelines.

Where sun exposure is insufficient to meet vitamin D needs, oral intake of the vitamin is an alternative source. Modern diets are generally low in vitamin D, and there is little food fortification in northern Europe. Therefore supplementation becomes the most efficient way to provide vitamin D through ingestion. We show how UV climatology can be used to inform the public about the potential need for vitamin D supplementation across the UK.

References

1. Webb AR, Kazantzidis A, Kift RC, Farrar MD, Wilkinson J, Rhodes LE (2018) Meeting Vitamin D Requirements in White Caucasians at UK Latitudes: Providing a Choice. Nutrients 10(4), 497; https://doi.org/10.3390/nu10040497
2. Webb, Ann R., Andreas Kazantzidis, Richard C. Kift, Mark D. Farrar, Jack Wilkinson and Lesley E. Rhodes (2018) Colour Counts: Sunlight and Skin Type as Drivers of Vitamin D Deficiency at UK Latitudes. Nutrients 10(4), 457; https://doi.org/10.3390/nu10040457

> OC100. Oral Communication
Symposium ENV-1 UV Monitoring for human health (Mario Blumthaler)

DATA FROM ENVIRONMENTAL UV MONITORING: WEIGHTING BY ACTION SPECTRA

Authors: Bjørn Johnsen[1], Tove Svendby[2], Arne Dahlback[3], Terje Christensen[1]
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The Norwegian UV-monitoring network, implemented in 1995/96 by the health and environmental authorities, has provided up to 24 years measurement data from nine stations located from 58°N to 78°N. Quality controlled data are now available for the general public and scientific community at https://github.com/uvnrpa.

The stations are equipped with GUV541 multiband filter radiometers from Biospherical Instruments Inc. (San-Diego), providing simultaneous measurements of global radiation every one minute throughout the years. Nominal peak wavelengths are 305, 313, 320, 340 and 380 nm, with 10 nm full width at half maximum. One station has an older GUV511 instead of a GUV541, where the only difference is a PAR channel (photosynthetic active radiation) instead of a 313 nm channel. Applying the methodology of Dahlback (App. Opt. 1996), geophysical factors like total ozone, cloud optical depth and surface albedo can be extracted from the measurement channels, providing key input parameters for the reconstruction of the solar UV spectrum.

A variety of action spectra, i.e. weighting functions for the relative biological effectiveness as a function of the wavelength of incident radiation, can be found in the literature. Doses weighted by eleven action spectra are currently available at github at one hour resolution. Data is freely available for non-profit use

The following weighted irradiance and dose products are included: Real sky skin erythema, total UVA and UVB, DNA-absorption, NMSC, vitamin D, fish eggs/embryos, plant growth, porphyria skin damage and PAR. Additionally, a complementary set of clear sky modelled irradiances and dose products are available, allowing the extraction of cloud modification factors. We encourage the use of these dose products for environmental and health effects studies. Users are recommended to reference the original action spectra from the literature to avoid ambiguity. One may develop unique, standardised names for the weighted dose products in the future. The global UV index is a good example of such a standardised weighted unit.

There is a need for more action spectra, particularly for health effects (e.g. skin damage), and terrestrial and aquatic ecosystems. More dose products will be added to github as soon as relevant action spectra become available. By weighting emission spectra of other sources than the sun, their risks and benefits can be directly compared with natural sunlight.

In summary:
• Complete datasets 1995-2018, measured and complemented for gaps in measurements
• Quality controlled – homogenized to a common irradiance scale
• Irradiance calibrations traceable to Qasume and the FARIN campaign.
• 11 dose products, 9 (10) stations

We declare no conflicts of interest.

> OC099. Oral Communication
Symposium ENV-1 UV Monitoring for human health (Mario Blumthaler)

MEASUREMENTS OF THE HEAD AND NECK EXPOSURE DISTRIBUTIONS OF SOLAR UV RADIATION – DEMANDS ON SKIN CANCER PREVENTION MEASURES

Authors: Peter M. Knuschke[1]
Presenting Author: Peter M. Knuschke
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Introduction

Basal cell carcinomas and squamous cell carcinomas (SCC) are widespread diseases. Annual solar UV-exposures more than twice higher in outdoor workers vs. the general population result in a twofold higher SCC risk. Since 2015 the SCC in high exposed outdoor workers is a recognised occupational disease in Germany. Between 2015 and 2017 7000 accepted skin cancer cases were registered in the building trade. Two third of the cases were located above the horizontal mouth angle axis. To compare the efficiency of skin protective measures in the head/neck region – for the general population as well as for outdoor workplaces (with or without mandatory safety helmets) – it is necessary to carry out detailed distribution measurements of the solar UV-exposure H_{er} [SED].

For the penetration of optical radiation (such as UVR) into the skin a cosine-like angular response is assumed. This has to take into account while evaluating the angular dependence of the incident solar radiation. Especially the so-called solar terraces of the skin are areas of increased skin cancer incidence. The polysulfone film (PSF) as actinic UV-sensor presents a good cosine response.

Methods

To simulate 8 hours moving under clear sky conditions 8 dummy heads were mounted on a carousel placed on top of a roof. Each dummy head was prepared with PSF-dosimeters at 14 positions – vertex of the head as reference. The 8h-exposures (9-17 MESZ) were carried out repeatedly at 3 days (averaging) in Jul. 2018 (solar noon elevation g_s = 60° (60°-day), UVI 7) and at 3 days in Sep. 2018 (g_s = 42° (42°-day), UVI 5). To investigate the skin protective effect against the solar UV-exposure 7 heads with caps/brimmed hats in comparison to an unprotected head were exposed. In a further study*) 7 heads with different types of safety helmets were investigated by identical protocol.

Results and Discussion

Relative to the vertex position the UV-exposures received on so-called sun terraces as bridge of the nose, ear helix or upper lip were higher at 60°-days vs. 42°-days. In contrast, the other (if unprotected) skin areas received higher relative UV-exposures at the 42°-days. The radiation penetrates more perpendicular to the skin and the sun-shielding components of caps, hats or helmets are less shadow effective.

Independent on the type of headgear, the lower skin areas as chin, upper lip, cheek or the neck (front and sides) are low protected. UV-exposure levels H_{er} per 8h between 7-24 SED at 60°-days or 4-12 SED at 42°-days (vs. 10-28 SED or 7-10 SED respectively for the unprotected head) mean multiples of the MED.

In result colour-coded overviews compare the UV-protective efficiency at 14 positions of the several headgear models at 60°- or 42°-days.

Conclusions

Even if UV-protective headgears will be used, an additional topical skin protection has to be applied.

Acknowledgement

supported by the German Social Accident Insurance Institution for the building trade

Conflicts of Interest:

no

> P108. Poster

Symposium ENV-1 UV Monitoring for human health (Mario Blumthaler)

UV-EXPOSURE OF YOUNG WOMEN FROM EVERY DAY LIFE CAUSED BY CLOTHING

Authors: Alois W. Schmalwieser[1], Veronika T. Schmalwieser[2], Susanne S. Schmalwieser[3]
Presenting Author: Alois W. Schmalwieser
1) Unit of Physiology and Biophysics, University of Veterinary Medicine, Vienna, Austria 2) BG Babenbergerring, Wiener Neustadt, Austria 3) BG Keimgasse, Mödling, Austria

Clothing is one of the most important factors for solar ultraviolet (UV) exposure of people. However, there is only little information on clothing habits available. Therefore, we investigated clothing of young females in dependence of meteorological parameters during every day live. Afterwards, we applied meteorological measurements and measurements of UV radiation to calculate the relative UV exposure of different body parts.

We developed a body chart which divides the body into six sections, together with a coding scheme that describes the worn garments. Clothing of more than 4000 women was observed in the urban region of Vienna and meteorological conditions were recorded.

Our study show that air temperature is the most important factor, while wind speed and humidity did not show any significant influence. Therefore we have generated frequency distributions for wearing certain garments in dependence of air temperature. Additionally, in temperatures from 10°C to 30°C, frequency of people was almost constant, but in higher temperatures, it decreased significantly.

The relative UV exposure of each body part was estimated using a) simultaneous measurements from the Austrian UV-Index monitoring network and air temperature over one year b) a model that recalculates the horizontally oriented measured irradiance to inclined planes c) clothing observations that provide the exposed body parts and with that the inclination of the skin and d) the relative frequency of people being outdoors for weighting.

We will show the UV exposure of different body parts throughout one year. Besides the face and hands, most exposed are the décolleté, ankles, instep and forearms because there are exposed already at lower temperatures. Further on, we demonstrate that air temperature and UV irradiance do not correlate well, so that both together with solar elevation are necessary to estimate UV exposure of people. With our results, an explanation for a recent skin status could be given.

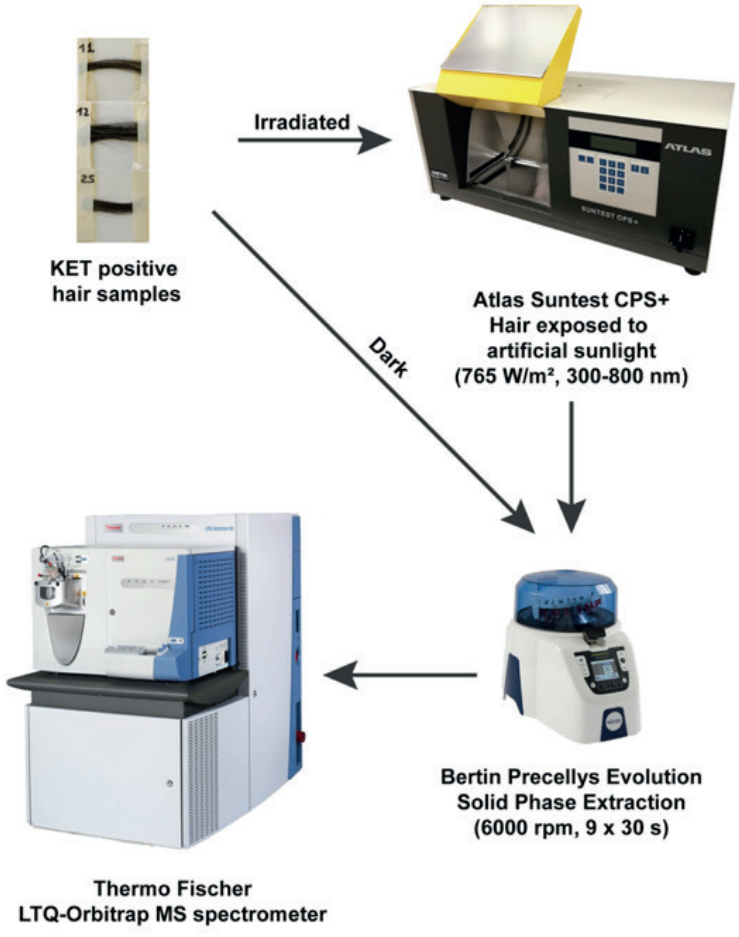
> P109. Poster

Symposium ENV-1 UV Monitoring for human health (Mario Blumthaler)

A PHOTOPRODUCT OF KETAMINE WAS IDENTIFIED IN HAIR SAMPLES IRRADIATED WITH ARTIFICIAL LIGHT IN A SOLAR SIMULATOR

Authors: Luca Menilli[1], Marianna Tucci[2], Giorgia Miolo[1], Donata Favretto[2]
Presenting Author: Luca Menilli
1) Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Italy 2) Legal Medicine and Toxicology, University Hospital of Padova, Italy

The main advantage of hair as a testing matrix is the ability to provide information related to historical drug exposure. Indeed, hair analysis has many applications within forensic (e.g., drug-related deaths, drug-facilitated crimes (DFCs), child protection) and clinical toxicology (e.g., drug rehabilitation programs, workplace drug testing). Exposure to sunlight and/or artificial light can induce photodegradation of licit/illicit drugs through photosensitization reactions. Therefore, when decisional cut-offs are applied to hair analysis (e.g., for granting a driving license, a job, or a child custody), it must be taken into account that hair exposed to sunlight may produce false results and lead to misjudgment. To better understand the role and the mechanisms of solar light on drugs in hair, the present work aims to evaluate the photodegradation of ketamine (KET) and its metabolite norketamine (NKET) in true positive hair samples irradiated in a solar simulator. KET and NKET concentration before and after irradiation were determined by means of liquid chromatography high-resolution mass spectrometry (HPLC-HRMS) in authentic hair samples from drugs users.



> P110. Poster

Symposium ENV-1 UV Monitoring for human health (Mario Blumthaler)

UVA-MONITORING IN THE EASTERN-ALPS

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UVA radiation has high biological impact. However, continuous monitoring is rarely done. In Austria, the existing UVB-Network was expanded several years ago by UVA-meters and data have been made available online. In the meanwhile, one station in Germany, two stations in Italy and two stations from Switzerland participate in the network.

Therefore, stations cover a wide range in elevation and represent typical geographical features like alpine upland, inner alpine valleys, high planes and mountains. The Austrian stations are situated in Vienna (16.43°E, 48.26°N, 153 m), Kirchbichl (12.09°, 47.49°N, 526m) and Mount Gerlitzen (13.9°E, 46.7°N, 1526 m). The stations in northern Italy stand in Leifers near Bozen (11.34°E, 46.43°N, 230 m) and Mount Ritten (11.43°E, 46.59°N, 1770 m). The Swiss stations can be found in Davos (9.83°E, 46.80°, 1610m) and at the close-by Mount Weissfluhjoch (9.82°E, 46.83°, 2540m). The station in Germany is located near the summit of Germany's highest mountain Mount Zugspitze (10.98°E, 47.42°N, 2667m).

At the German station the solar UV irradiance is measured spectrally resolved with a BTS diode array radiometer. The other stations are equipped with broadband meters from different manufacturers, which respond approximately the wavelength range of 310 nm to 400 nm and possess a cosine-like response. Measurements are corrected by a calibration matrix in respect to solar elevation and total ozone column (as there sometimes is a noticeable sensitivity in the UVB). At some of the stations both global and diffuse (using a shadow-ring or a sun tracker together with a shadow-ball) UVA radiation is measured. The devices are cared according to international recommendations for UV-Index measurements.

Here we present the global and diffuse UVA measurements over several years as well as a data analysis. The altitude effect is estimated to be 8%-9% per 1000 m. Seasonal differences in cloud cover result in higher UVA radiation in the first half of the year, than in the second half. Further differences in cloud cover, like very low cloudiness in the flat plains (e.g. Vienna) during the summer lead to higher UVA radiation exposure than measured in the mountainous regions (e.g. Mt. Gerlitzen).

Our presentation will show that there are obvious differences in UVA radiation within the alpine region, which are not only traceable to topography, but also to local climate.

> P111. Poster

Symposium ENV-1 UV Monitoring for human health (Mario Blumthaler)

FIRST RESULTS FROM PERSONAL UV EXPOSURE MEASUREMENTS IN KENYA

Authors: Dagmar Schoder[1], Jakob Heydenreich [2], Alois W. Schmalwieser[3]
Presenting Author: Dagmar Schoder
1) Institute of Milk Hygiene, Univ. of Veterinary Medicine, Vienna, Austria & Veterinaires sans frontiers Austria 2) Bispebjerg Hospital, Copenhagen, Denmark 3) Institute of Physiology and Biophysics, Univ. of Veterinary Medicine, Vienna, Austria

During the past 40 years, a variety of studies have been undertaken to measure the personal UV exposure of people. Almost all studies focused on light skinned populations in Europe, Australia and North America. Until today, no measurements have been made at the Equator, where UV radiation is highest.

For this study, personal UV exposure measurements were made at two different locations in Kenya: Malindi, located at the coast and Nairobi, located at an altitude of 1660 m asl. A variety of volunteers with different professions have been equipped with wrist-watch-like electronic devices, which measure the erythemally effective UV irradiation at the wrist. These devices have been calibrated to solar radiation prior to the study and calibration was checked after the study.

As one of the interesting results, the differences in personal UV exposure between the left and the right hand of a taxi driver due to the open car window could be quantified. We will show that the open car window has s significant influence on UV exposure.

Most exposed are guards during their inspection gallery and gardeners, while street vendors could choose shaded places and therefore are less exposed.

Beside others, we will show that the “Exposure ratio to ambient UV” for gardeners in Kenya is similar to that for gardeners in Europe and that the median personal UV exposure is close to 1 MED for skin type VI in Kenya.

> IL218. Invited Lecture

Symposium ENV-2 UV radiation and rapid climate change: implications for plants and ecosystems
(Janet Bornman Yolanda Solà)

CLIMATE CHANGE, OZONE AND UV RADIATION: WHERE WE ARE AND WHAT WE MIGHT EXPECT

Authors: Ann Webb[1]
Presenting Author: Ann Webb
1) University of Manchester

Changes in our atmosphere, on timescales that can be measured in decades, are influencing the climate and environment experienced at the surface of the earth. Changes in greenhouse gases (GHGs) and ozone depleting substances (ODSs) are commonly associated with global warming and ozone depletion/recovery, respectively, but their influences are not entirely independent of each other. Both influence the UV radiation environment that we might expect to receive in the future.

In addition to column ozone, the major determinants of UV radiation at the surface are clouds, aerosols and surface reflectivity, all of which are changing or expected to change in response to GHG induced climate change, and human activity. The net effect of ozone recovery, these other influences and their interactions, becomes complex and is location and season dependent. For example, Chemistry-Climate Models using representative GHG concentration scenarios show that projected ozone recovery dominates end-of-century UV in the Antarctic, while in east Asia changes in aerosols have by far the greatest impact (Bais et al., 2018).

Further global projections from the UNEP Environmental Effects Assessment Panel will be used to illustrate the anticipated UV environment of the future, while long-term measurements from the UK will show how ozone and UV have changed to date in a region without extreme atmospheric conditions.

References

A. F. Bais, G. Bernhard, R. L. McKenzie, P. J. Aucamp, P. J. Young, M. Ilyas, P. Jöckel and M. Deushi Ozone–climate interactions and efects on solar ultraviolet radiation. Photochem. Photobiol. Sci., 2019,18, 602–640

> IL222. Invited Lecture

Symposium ENV-2 UV radiation and rapid climate change: implications for plants and ecosystems
(Janet Bornman Yolanda Solà)

OBSERVED INFLUENCE OF CLOUD PROPERTIES ON ULTRAVIOLET SURFACE RADIATION

Authors: David Mateos[1], J. Bilbao[1], A. di Sarra[2], A. de Miguel[1], G. Pace[2], D. Meloni[2], G. Casasanta[3], Q. Min[4], V.E. Cachorro[1], A. de Frutos[1]
Presenting Author: David Mateos
1) Universidad de Valladolid 2) ENEA-UTMEA Ter 3) Institute of Atmospheric Sciences and Climate 4) Atmospheric Sciences Research Center

UV radiation exerts a significant influence on the biosphere and atmospheric chemistry, and its propagation through the atmosphere is strongly modulated by clouds. Few experimental studies on the cloud effects on UV solar radiation have been carried out so far, due to the lack of simultaneous measurements of UV radiation and cloud properties, which show a large temporal and spatial variability. Only in recent years new experimental and theoretical methods have been applied to this topic. UV irradiance is usually represented with the UV index, UVI, which describes the UV radiation levels at the surface that produce erythema or sunburn on human skin. One key reaction in the chemistry of the troposphere is ozone photolysis, for which the rate of this reaction, J(O1D), is used.

A large number of instruments located at three different European stations were involved in the analysis described in this study. These instruments provided measurements of global and diffuse spectral irradiances and spectral actinic flux in the UV range, UVI, J(O1D), cloud optical thickness, liquid water path, effective radius of cloud droplets, total ozone column, cloud cover, cloud base and top heights, and aerosol optical thickness (as well as its vertical distribution). Radiative transfer simulations with the libRadtran library have been carried out as realistically as possible.

Most experimental and modeling studies on this topic used the cloud modification factor (CMF), which is defined as $UV_{cloudy}/UV_{cloud\text{-}free}$, where UV_{cloudy} and $UV_{cloud\text{-}free}$ are the UV radiation under cloudy and cloud-free conditions, respectively, for the same atmospheric conditions. The CMF can be evaluated for spectrally dependent or spectrally integrated quantities and for different radiative quantities (irradiance, weighted irradiance, actinic flux, and photolysis rate). Various studies have shown that the CMF for the UV irradiance displays a wavelength dependence, with a higher cloud transmission at 320 than at 400nm. This dependency is attributed to molecular scattering occurring above the cloud layer; the same effect is observed at high values of solar zenith angle (SZA), when the diffuse to direct ratio is large. On the other hand, attenuation at the shorter wavelengths is due to enhanced absorption by tropospheric ozone. This study contributes to the characterization of the UV radiative flux under overcast conditions by investigating the role of cloud optical and microphysical properties on UV index, ozone photolysis rate, global and diffuse spectral irradiance and spectral actinic flux at the surface under overcast conditions.

> IL221. Invited Lecture

Symposium ENV-2 UV radiation and rapid climate change: implications for plants and ecosystems
(Janet Bornman Yolanda Solà)

SOLAR ERYTHEMAL IRRADIANCE FROM SATELLITE AND GROUND-BASED INSTRUMENTS: THE INFLUENCE OF CLOUDINESS

Authors: Yolanda Sola[1], Joan Bech[1]
Presenting Author: Yolanda Sola
1) Group of Meteorology, Dpt. Applied Physics, University of Bacelona

The variability and trends in surface solar ultraviolet (UV) irradiance have become of great interest during the last decades given the potentially harmful effects on humans, such as the erythema and skin cancer, as well as the ozone depletion observed from the 1980s. As a result, the number of stations making regular measurements of UV and erythemal irradiances has increased and spread out. Ground-based measurements represent high temporal resolution, although the spatial coverage is still sparse and irregularly distributed. The large spatial coverage of data derived from sensors onboard of satellite platforms represent an important advantage. However, satellite data require continuous comparisons with high-quality ground-based observations to assure the quality of satellite UV products. Cloudiness has great influence on the short-term variability of the surface solar irradiance and its role is even more relevant in the UV erythemal irradiance. In the comparison of ground-based and satellite date, clouds also represent a key factor. The characterization of cloudiness is complex since it entails a large number of parameters related both to microphysical properties (i.e., droplet size distribution) and to macrophysical properties, such as the percent cover and the cloud-sky configuration. Moreover, clouds also affect the determination of UV irradiances from satellite observations.

We have compared ground-based UV erythemal irradiance with satellite-based UV products of the Ozone Monitoring Instrument (OMI) at solar noon. Measurements were performed in Barcelona (41.35N, 2.16 E) with a YES UVB1 broadband radiometer belonging to the Spanish Meteorological Agency (AEMET) network. The cloud cover and types are based on routine visual observations from Fabra Observatory, close to the radiometers. Complementary, we have derived atmospheric conditions from the modified clearness index, which represents the attenuation of the solar radiation through the atmosphere and, in first order it is representative of the cloudiness effects. The clearness index was determined from 10-min averaged values of global horizontal irradiance measured by a Kipp&Zonen CM-11 pyranometer. This long time series were previously analyzed by Bech et al. (2015).

To ensure clear-sky conditions for both datasets, we have selected 30-min mean values of surface erythemal irradiance at 12 UTC satisfying that the observed cloud cover at 13 UTC was 0 okt and the averaged clearness index (11:30-13:30UTC) was higher than 0.75. It is observed that the OMI product overestimates the surface erythemal irradiance, especially in winter months with an annual relative error of 19%. This bias is higher when the comparison is based on measurements under overcast conditions.

Reference

Bech, J., Sola, Y., Ossó, A., Lorente, J., 2015, Analysis of 14 years of broadband ground-based solar UV index observations in Barcelona. Int. J. Climatol., 35(1), 45-56, doi:10.1002/joc.3961.

We would like to thank the Spanish Meteorological Agency, the Fabra Observatory and the OMI scientific team for providing the different datasets used in this study.

> IL220. Invited Lecture

Symposium ENV-2 UV radiation and rapid climate change: implications for plants and ecosystems
(Janet Bornman Yolanda Solà)

ENVIRONMENTAL EFFECTS OF OZONE-DRIVEN CLIMATE CHANGE IN THE SOUTHERN HEMISPHERE

Authors: Sharon Robinson[1]
Presenting Author: Sharon Robinson
1) University of Wollongong

Stratospheric ozone depletion has been a major driver of Southern Hemisphere climate change over the later part of the 20thCentury. In particular, the zone of strong westerly winds has shifted south influencing both ocean circulation and regions of rainfall, with reduced precipitation in mid latitudes and enhanced precipitation in the subtropics. The implications of these changes in climate are likely to be far more pervasive for both terrestrial and marine ecosystems than the increase in ultraviolet-B radiation due to ozone depletion; however, they tend to be overlooked in the biological literature. In this talk I will synthesize our understanding of how this ozone-linked climate change has affected terrestrial and marine ecosystems[1-3]. The largest impacts are found in the Southern Hemisphere summer season (December–February). The ecosystem impacts documented so far include changes to growth rates of South American and New Zealand trees, decreased health of both Antarctic mosses4 and sub Antarctic cushion plants[5] and changing biodiversity in Antarctic lakes. Given the extent of changes to climate across the Southern Hemisphere, they are likely to have had as much or more impact on natural ecosystems and food production over the past few decades, as the increase in ultraviolet radiation due to ozone depletion.

Refences

1. Robinson, S. A. and D. J. Erickson (2015). «Not just about sunburn – the ozone hole\›s profound effect on climate has significant implications for Southern Hemisphere ecosystems.» Global Change Biology**21**(2): 515-527.
2. Bornman, J., et al. (2019). “Linkages between stratospheric ozone, UV radiation and climate change and their implications for terrestrial ecosystems.” Photochemical & Photobiological Sciences**18**: 681-716.
3. Williamson, C. E., et al. (2019). “The interactive effects of stratospheric ozone depletion, UV radiation, and climate change on aquatic ecosystems.” Photochemical & Photobiological Sciences **18**(3): 717-746.
4. Robinson, S. A., et al. (2018). “Rapid change in East Antarctic terrestrial vegetation in response to regional drying.” Nature Climate Change**8** 879-884.
5. Bergstrom, D. M., et al. (2015). “Rapid collapse of a sub-Antarctic alpine ecosystem: the role of climate and pathogens.” Journal of Applied Ecology**52**(3): 774-783.

> IL215. Invited Lecture

Symposium ENV-2 UV radiation and rapid climate change: implications for plants and ecosystems
(Janet Bornman Yolanda Solà)

THE IMPLICATIONS FOR PLANT ECOLOGY OF ALTERED UV EXPOSURE IN A CHANGING CLIMATE

Authors: T. Matthew Robson[1], Craig C. Brelsford[1], Marta Pieristè[1,2], Saara M. Hartikainen[1], Twinkle Solanki[1]
Presenting Author: T. Matthew Robson
1) Organismal and Evolutionary Biology (OEB), Viikki Plant Science Centre (ViPS), Faculty of Biological and Environmental Science, P.O. Box 65, 00014, University of Helsinki, Finland. 2) Normandie Université, UNIROUEN, IRSTEA, ECODIV, FR Scale CNRS 3730, Rouen, France

It is more than 30-years since the signing in 1987 of the Montreal Protocol which restricted the production of ozone-depleting chemicals and led researchers to question the role of UV radiation in shaping terrestrial ecosystems. As well as reversing the increase in size of the southern hemispheric ozone hole, the limits imposed by the Montreal Protocol have to-date stopped an ozone hole from developing over the Arctic (Bornman et al, 2019). Nevertheless, complex interactions between the polar climate, ocean currents, and stratospheric-ozone depletion leave the potential for unpredictable conditions to affect regions at high latitudes. The likely outcomes are expected to include warming temperatures, changes in patterns of precipitation and thus solar radiation, and more-frequent extreme climatic events.

I will present research into plant responses to changes in their light environment and its spectral composition, using examples from plant communities in Finland that exemplify broader global patterns of response. Reduced snow cover will expose plants to fluctuating temperatures and irradiances during winter and early spring, potentially altering their phenology and interfering with dehardening when they start to photosynthesize. Plant photoreceptors for UV and blue light mediate the accumulation of phenolic UV-screening compounds which also function as antioxidants in leaves (Brelsford et al, 2019). This knowledge of the mechanisms of plant response, allows us to better forecast the effects on plant ecology of the changes in spectral quality that occur across latitudes, seasons and with leaf-out in deciduous forest canopies (Hartikainen et al, 2018). In particular, the interaction between temperature and photoreceptor-mediated responses affecting photoprotection will determine how well plant species cope with earlier snow melt in spring (Solanki et al, 2019).

The broader context of this research includes considering how ecosystem processes, such as litter decomposition, are being affected by changes in the growing season length. These are complex processes governed by multiple biotic and abiotic controls: e.g. photodegradation depends on structural, biochemical, and optical leaf traits responding to the light environment during plant growth and decomposition, and on interactions with the leaf and soil microbial communities. Temperate species range-shifts may also reach a northern limit in Finland because of the restrictions imposed by season patterns of day length and solar irradiance (Brelsford & Robson, 2018).

References

Bornman JF et al (2019) *Photochemical & Photobiological Sciences* 18 681-716. https://doi.org/10.1039/C8PP90061b
Brelsford et al (2019) *Physiologia Plantarum* https://doi.org/10.1111/ppl.12749
Brelsford CC, Robson TM (2018) *Trees Structure & Function* 32, 1157-1164 https://doi.org/10.1007/s00468-018-1684-1
Hartikainen SM, et al (2018) *Ecology & Evolution* 8, 10206-10218. https://doi.org/10.1002/ece3.4496
Solanki T, et al (2019) *Plant Physiology and Biochemistry* 134, 40-52. https://doi.org/10.1016/j.plaphy.2018.09.003

> IL217. Invited Lecture

Symposium ENV-2 UV radiation and rapid climate change: implications for plants and ecosystems
(Janet Bornman Yolanda Solà)

PHYSIOLOGICAL PLASTICITY COMPLEMENTS THE GENETIC ADAPTATION OF GRAPEVINE LEAVES TO SOLAR RADIATION*

Authors: Éva Hideg[1]
Presenting Author: Éva Hideg
1) Department of Plant Biology, University of Pécs, Hungary

Plant responses to climate factors comprise two major components, long-term-adaptive responses and short-term adjustments of physiology in response to rapid changes. Results of two experiments illustrate this.

Long-term adaptive responses were analyzed using sun-acclimated grapevine (Vitis vinifera cv. Pinot noir) leaves collected from twelve locations across a 36.69 - 49.98°N latitudinal gradient in Europe. Leaves were collected at the onset of berry ripening (veraison) and metabolic profiles were explored in connection to meteorological parameters which characterized each sampling site. We found that cumulative UV radiation, which leaves received during the 3-4 months between bud break and leaf collection, was the strongest correlator with most metabolites and pigments. Leaf UV-absorbing pigments, total antioxidant capacities, and phenolic compounds increased with increasing cumulative UV. On the other hand, total carotenoids and xanthophylls decreased with increasing cumulative UV.

In the second experiment, rapid, hourly changes in leaf phenolic contents of the same grapevine cultivar were studied at one location (46º07' N, 18º17'E) at one time point, at the time of veraison. This study showed that on top of phenolic profiles built up in leaves as long-term acclimation to local climate conditions, a specific small fraction of compounds responded to dynamic changes in the natural environment. In addition to solar radiation, leaf temperature was also identified as a positive correlator of epidermal UV absorbance. Total flavonoid content, on the other hand, showed no statistical connection to these parameters but was positively correlated to air temperature.

These two studies show that physiological plasticity, and especially metabolic plasticity, complement plant genetic adaptations; and emphasize the role of phenolic compounds, in grapevine leaves these are glycosylated quercetins, in both long- and short-term acclimation.

Contributed by co-authors of the following publications:

Castagna, A., Csepregi, K., Neugart, S., Zipoli, G., Večeřová, K., Jakab, G., Jug, T., Llorens, L., Martínez-Abaigar, J., Martínez-Lüscher, J., Núñez-Olivera, N., Ranieri, A., Schoedl-Hummel, K., Schreiner, M., Teszlák, P., Tittmann, S., Urban, O., Verdaguer, D., Jansen, M.A.K., Hideg, É. (2017) Environmental plasticity of Pinot noir grapevine leaves; a trans-European study of morphological and biochemical changes along a 1500 km latitudinal climatic gradient. *Plant Cell and Environment* 40, 2790-2805.

Csepregi, K., Teszlák, P., Kőrösi, L., Hideg, É. (2019) Changes in grapevine leaf phenolic profiles during the day are temperature rather than irradiance driven. *Plant Physiology and Biochemistry* 137, 169-178.

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> IL216. Invited Lecture

Symposium ENV-2 UV radiation and rapid climate change: implications for plants and ecosystems
(Janet Bornman Yolanda Solà)

HORMONE RESPONSES TO SHORT DAILY UV-B IRRADIATION IN TOMATO PLANTS: WHAT HAPPENS IN THE UPPER AND BELOW-GROUND ORGANS?

Authors: Alessia Mannucci[1], Lorenzo Mariotti[1], Rodolfo Bernardi[1], Alice Trivellini[2], Thais Huarancca Reyes[1], Anna Mensuali[2], Annamaria Ranieri[1], Marco Santin[1], Antonella Castagna[1], Mike Frank Quartacci[1]
Presenting Author: Alessia Mannucci
1) Department of Agriculture, Food and Environment, University of Pisa, Italy. 2) Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy.

UV-B radiation is known to influence many aspects of plant physiology and biochemistry through a signalling route triggered by UV-B perception by the specific photoreceptor UVR8. However, most of these studies were mainly addressed to investigate the behaviour of the above-ground organs since they are directly exposed to the solar UV-B radiation (Ulm et al., 2004; Brown & Jenkins, 2008). Little is instead known about the root responses, despite UV-B radiation affecting the morphology of this organ as well. This suggests a perceiving mechanism also in the roots and/or a shoot-to-root signalling transmission (Tong et al., 2008; Leasure et al., 2009).

The present research aimed to understand whether low doses of UV-B radiation (1.19 KJ/m^2 per day, 15 min a day, using narrow-band lamps) applied above-ground influenced the hormonal balance in both leaves and roots of Micro-Tom tomato (*Solanum lycopersicum* L.) plantlets. Twenty-five-day-old plantlets received daily UV-B irradiation for 11 days under controlled conditions (PPFD 228 µmol m^{-2} s^{-1}, 80% R.H., 22°C). Changes in hormone level, including ethylene, abscisic acid, indoleacetic acid and salicylic acid, were monitored at 8 (UV_8) and 11 (UV_{11}) days of UV-B treatment and 3 days after the end of irradiation (UV_{11+3}). Gene expression of the enzymes involved in ethylene biosynthesis was investigated by qRT-PCR. Photosynthesis performance was monitored by non-destructive techniques to ensure that the UV-B dose was not stressful for the plantlets. Finally, H_2O_2 and $O_2^{\cdot-}$ content and the antioxidant activity were evaluated as markers of possible UV-B-induced oxidative stress.

The irradiated leaves of Micro-Tom displayed a significant decrease in ethylene emission of 38% and 42% after 8 days and 11 days respectively, confirming a previous report on the UVR8-mediated down-regulation of ethylene biosynthesis (Hectors et al., 2007). However, this decrease was transient since ethylene emission of UV_{11+3} was similar to the control. Roots of UV-B treated samples responded differently to leaves with respect to ethylene emission.

Abscisic, indoleacetic and salicylic acid levels in leaves were different depending of the irradiation period (UV_8 and UV_{11}) with respect to the control and they were transient in some cases, since hormone levels in UV_{11+3} samples were similar to the control. Levels of these plant hormones were also evaluated in roots.

All these results revealed an intricate UV-B response mechanism between above- and below-ground organs which will be discussed.

References

Brown, B. A., & Jenkins, G. I. (2008). Plant Physiol, 146(2), 576-588.
Hectors, K., et al. (2007). New Phytol, 175(2), 255-270.
Leasure, C. D., et al. (2009). Plant Physiol, 150(4), 1902-1915.
Tong, H., et al. (2008). PNAS 105(52), 21039-21044.
Ulm R., et al. (2004). PNAS 101, 1397–1402.

> IL219. Invited Lecture

Symposium ENV-2 UV radiation and rapid climate change: implications for plants and ecosystems
(Janet Bornman Yolanda Solà)

LIGHT POLLUTION INDICATORS TO TRANCE THE ENVIRONMENTAL IMPACT

Authors: Alejandro Sánchez de Miguel[Unive]
Presenting Author: Alejandro Sánchez de Miguel
1) University of Exeter

Light pollution is a big growing problem, but as a new discipline, we have not yet agreed on an indicator or collection of indicators that represent on a straight way the environmental impact of the light pollution. Some times is impossible to simplify the complexity of the problem so, a collection of the different indicators and ways of measuring them on the most simple way will be presented showing the state of the art of the measurements of the light pollution

> P112. Poster

Symposium ENV-2 UV radiation and rapid climate change: implications for plants and ecosystems
(Janet Bornman Yolanda Solà)

EFFECTS OF UVB-COMBINED WITH GAMMA RADIATION DURING EARLY LIFE STAGES OF AN EXPERIMENTAL MODEL FISH, THE ZEBRAFISH (DANIO RERIO)

Authors: Selma Hurem[1,3], Terje Christensen[2,3], Jan L. Lyche[1,3], Peter Aleström[1,3]
Presenting Author: Terje Christensen
1) Norwegian University of Life Sciences (NMBU), Faculty of Veterinary Medicine and Biosciences 2) Norwegian Radiation and Nuclear Safety Authority 3) Centre for Environmental Radioactivity (CERAD CoE)

Rapid changes in the climate are occurring, which may lead to a change in UV radiation because of variations in the thickness of the Ozone layer, reduced snow and ice cover, thereby causing lower albedo (reflection of solar radiation) at certain times of the year, as well as increased or decreased cloud cover.

At the same time, co-exposure to UV and ionizing radiation above the natural levels may take place in critical environments, like in the Barents Sea, Chernobyl or Fukushima, and may become more probable in the future.

The aim of this study was to assess potential synergistic or antagonistic effects of gamma radiation on zebrafish embryos when combined with simultaneous exposure to environmentally relevant UV radiation doses.

We exposed zebrafish embryos to gamma radiation from a ^{60}Co source, activity ~ 420 GBq with the following dose rates: 0, 10 and 40 mGy/h at 2 – 5.5 hours post fertilization (hpf). The gamma irradiation was interrupted for 5 min at 4.5 hpf for exposure of the embryos to sub-lethal and environmentally relevant doses of UV-B radiation from broadband fluorescent tubes (Philips PI 12, 0.42 mW/cm^2).

Behaviour effects, physiological effects, ROS, lipid peroxidation, mortality and malformations were assessed as well as effects on the transcriptome.

A UV-B-dose dependent lowering of the heart rate was observed at 50 hpf, which did not persist at 60 hpf. A locomotor assay taking advantage of a high throughput image analysis system was performed at 96 hpf. The highest dose of UV-B radiation led to an increase in the time spent active and a slower average swimming speed although these effects were not significant ($p = 0.07$). UV-B exposures also caused effects on ROS formation and lipid peroxidation.

Gene expression analyses (RNAseq) of the larvae exposed to gamma radiation indicate a dose-response relationship between the numbers of differentially regulated genes compared to controls. The differentially regulated genes in the low dose rate group formed functional networks involved in retinoic acid receptor activation (RARa), apoptosis and glutathione-mediated detoxification signalling pathways as the most affected. The most affected signalling pathways in higher dose rate groups were eif2, eif4/p70s6k and mTOR, which is i.a. involved in the modulation of angiogenesis. Further work includes RNA analyses of embryos treated by the combination of UV-B and gamma radiation. No conflicts of interest

> IL223. Invited Lecture
Symposium ENV-3 Vitamin D (Mark Farrar)

INTERRELATIONSHIPS BETWEEN DIETARY VITAMIN D, EXPOSURE TO UV RADIATION AND THE FAECAL MICROBIOME

Authors: Prue Hart[1], Simon Ghaly[1, 2], Nadeem Kaakoush[3]
Presenting Author: Prue Hart
1) Telethon Kids Institute, University of Western Australia, Perth, WA, Australia 2) Department of Gastroenterology and Hepatology, St. Vincent's Hospital, Sydney, NSW, Australia 3) School of Medical Sciences, University of NSW Sydney, Kensington, NSW, Australia

Introduction
The intestinal microbiota plays an important role in development of the immune system and regulation of immune responses not only in the gut but also in distant vascularised tissues such as the central nervous system. The health of gastrointestinal tract is dependent on the bi-directional interaction between gut microbial antigens and the intestinal immune system to maintain homeostasis or "physiological inflammation". Reduced sunlight exposure has been associated with an increased incidence of Crohn's disease and Ulcerative Colitis but the effect of ultraviolet radiation (UVR) on the faecal microbiome and susceptibility to colitis has not been explored. By a comparison with the effect of different vitamin D-containing diets, our study investigated the effect of UVR by both vitamin D-dependent and -independent pathways.

Methods
C57Bl/6 female mice were fed three different vitamin D-containing diets for 24 days before half of the mice in each group were UV-irradiated (1 kJ/m^2) for each of 4 days, followed by twice weekly irradiation of shaved dorsal skin for 35 days. Faecal DNA was extracted and high-throughput sequencing of the 16S RNA gene performed.

Results and Discussion
UV-irradiation of skin was associated with a significant change in the beta-diversity of faeces compared to non-irradiated mice, independently of vitamin D. Specifically, members of phylum *Firmicutes*, including *Coprococcus* were enriched, whereas members of phylum *Bacteroides*, such as *Bacteroidales* were depleted. Expression of colonic *CYP27B1* increased by 4-fold and *IL1b* decreased by 5-fold, suggesting a UVR-induced anti-inflammatory effect. UV-irradiated mice, however, were not protected against colitis induced by dextran sodium sulfate (DSS), although distinct faecal microbiome differences were documented post DSS between UV-irradiated and non-irradiated mice.

Conclusions
Both vitamin D diets and skin exposure to UVR altered the faecal microbiome. Several vitamin D-dependent and -independent pathways by which UVR may suppress immunity have been suggested, and may include an effect on the faecal microbiome which in turn may control the development of immune cell subsets with different biological activities.

Reference

Ghaly et al., Ultraviolet irradiation of skin alters the faecal microbiome independently of vitamin D in mice. *Nutrients* **2018**, 10, 1069; doi:10.3390/nu10081069

> IL224. Invited Lecture
Symposium ENV-3 Vitamin D (Mark Farrar)

PHOTOPROTECTION BY CALCITRIOL (1,25DIHYDROXYVITAMIN D) AND RELATED COMPOUNDS.

Authors: Rebecca S. Mason[1, 2], Katie M. Dixon[1, 3], Mark S. Rybchyn[1, 2]
Presenting Author: Rebecca S Mason
1) Bosch Institute, University of Sydney 2) Physiology, University of Sydney 3) Anatomy & Histology, University of Sydney

Exposure of skin cells to UV radiation results in DNA damage, which if inadequately repaired, may cause mutations. UV-induced DNA damage as well as reactive oxygen and nitrogen species also cause local and systemic suppression of the adaptive immune system. Together these changes underpin the development of skin tumours. The hormone derived from vitamin D, calcitriol (1,25-dihydroxyvitamin D), and other related compounds, working via the vitamin D receptor and at least in part, through endoplasmic reticulum protein 57 (ERp57), reduce cyclobutane pyrimidine dimers and oxidative DNA damage in keratinocytes and other skin cell types after UV. Calcitriol and related compounds enhance DNA repair in keratinocytes, in part through decreased reactive oxygen species, increased p53 expression and/or activation, increased repair proteins and in part through increased energy availability in the cell when calcitriol is present after UV exposure. Oxidative phosphorylation is suppressed in keratinocytes exposed to UV. In the presence of calcitriol, but not vehicle, glycolysis is increased after UV, along with increased energy conserving autophagy and changes consistent with enhanced mitophagy. Reduced DNA damage and reduced ROS/RNS should help reduce UV-induced immune suppression. Reduced UV-immune suppression is observed after topical treatment with calcitriol and related compounds in mice. These protective effects of calcitriol and related compounds presumably contribute to the observed reduction in skin tumor formation in mice after chronic exposure to UV followed by topical treatment with calcitriol and some, though not all, related compounds.

> IL225. Invited Lecture
Symposium ENV-3 Vitamin D (Mark Farrar)

SEASONAL SUNLIGHT EXPOSURE AND VITAMIN D STATUS IN OLDER ADULTS

Authors: Mark Farrar[1], Richard Kift[1], Kevin Cashman[2], Ann Webb[1], Lesley Rhodes[1]
Presenting Author: Mark Farrar
1) University of Manchester 2) University College Cork

Sunlight exposure of skin is the major source of vitamin D, which is essential for musculoskeletal health. Vitamin D status may be compromised in older adults through reduced capacity of skin to produce vitamin D, but we do not know the sunlight exposure levels and vitamin D status of the growing number of ≥65 year-olds in the UK and how this might differ from younger adults. We performed a prospective cohort study to assess this and potential contributory factors in adults aged ≥65 years, with comparison to our previous study in younger adults (20-60 years)[1].

Healthy white Caucasian adults (n=127; median age 71 years, range 65-85) were recruited in Greater Manchester, UK. Circulating 25-hydroxyvitamin D (25OHD), personal UVR dose levels and dietary vitamin D intake were assessed at summer-end (September) and in winter (January). Personal UVR dose was measured using polysulphone film badges with one badge worn on weekdays and a separate badge worn at weekends. Dietary intake was determined through completion of daily food logs.

Mean (SD) 25OHD was 64.8 (21.8) nmol/L in September with 3% deficient (25OHD <25 nmol/L) and 21% insufficient (25 to <50 nmol/L). In January, mean (SD) 25OHD was 53.6 (24.1) nmol/L with 10% deficient and 46% insufficient. Dietary vitamin D intake was low (<5 µg/day) and did not differ between seasons. Daily personal UVR doses received in September were equivalent on weekdays and weekend days with a median (IQR) UVR dose of 0.60 (0.29 – 1.19) SED/day on weekdays and 0.64 (0.24 – 1.59) SED/day on weekend days. Compared to younger adults (20-60 years), the older adult cohort exhibited a narrower range of 25OHD levels and higher prevalence of vitamin D deficiency.

Sun-exposure levels of white Caucasian adults aged 65 years and over in the UK were not able to provide adequate vitamin D, with approximately one-quarter having 25OHD <50 nmol/L at summer-peak. Increased awareness of vitamin D sources and further national guidance may be required for this age group.

Reference

1. Webb AR, Kift R, Durkin MT, O'Brien SJ, Vail A, Berry JL, Rhodes LE. The role of sunlight exposure in determining the vitamin D status of the UK white adult population. Br J Dermatol 2010;163:1050-55.

> IL226. Invited Lecture
Symposium ENV-3 Vitamin D (Mark Farrar)

OPTIMAL SUNSCREEN USE, DURING A SUN-HOLIDAY WITH A VERY HIGH UV INDEX, ALLOWS VITAMIN D SYNTHESIS WITHOUT SUNBURN

Authors: Antony Young[1], Peter Philipsen[2]
Presenting Author: Antony Young
1) King's College London, London, UK 2) Bispebjerg Hospital, Department of Dermatology, Copenhagen, Denmark

Introduction
Sunlight contains UVA and UVB radiation. The latter is essential for vitamin D synthesis but is the main cause of sunburn and skin cancer. Sunscreen use is advocated to reduce the sun's adverse effects but may compromise vitamin D status.

Methods
The impact of sunscreens on vitamin D status was studied during a one-week sun-holiday in Tenerife (28°N). Comparisons were made between two formulations, each with a sun protection factor of 15. The UVA protection factor (UVA-PF) was low in one case and high in the other. Healthy Polish volunteers (n=20 per group) were given the sunscreens and advised on correct application. Comparisons were also made with discretionary sunscreen use (n=22) and non-holiday groups (51.5°N, n=17). Sunscreen use in the intervention groups was measured. Behaviour, personal UVR exposure, clothing cover and sunburn were monitored. Serum 25(OH)D, was assessed by HPLC MS/MS.

Results and Discussion
Use of intervention sunscreens was the same ($p=0.599$) with a mean application thickness of 2.4mg/cm^2, and both equally inhibited sunburn, that was present in the discretionary use group. There was an increase ($p=9\times10^{-8}$) of 28.0±16.5(SD) nmol/L 25(OH)D_3 in the discretionary use group. The high and low UVA-PF sunscreen groups showed statistically significant increases ($p\leq6.7\times10^{-5}$) of 19.0±14.2 and 13.0±11.4 nmol/L 25(OH)D_3 respectively. The non-holiday group showed a fall ($p=0.08$) of 2.5±5.6 nmol/L 25(OH)D_3.

Conclusions
Sunscreens may be used to prevent sunburn yet allow vitamin D synthesis. A high UVA-PF sunscreen enables significantly higher vitamin D synthesis than a low UVA-PF sunscreen because the former, by default, transmits more UVB than the latter.

Acknowledgements
This study was funded by the EC and Walgreens Boots Alliance Inc.

References

• J. Narbutt, PA Philipsen, GI Harrison, KA Morgan, KP Lawrence, KA Baczynska, K. Grys, M Rogowski-Tylman, I Olejniczak-Staruch, A Tewari, M Bell, C O\'Connor, HC Wulf, A Lesiak, AR Young (2019). Sunscreen applied at ≥ 2 mg cm^{-2} during a sunny holiday prevents erythema, a biomarker of ultraviolet radiation-induced DNA damage and suppression of acquired immunity. Br J Dermatol, 180: 604–614.
• AR Young, J Narbutt, GI Harrison, KP Lawrence, M Bell, C O'Connor, P Olson, K Grys, KA Baczynska, M Rogowski-Tylman, H Wulf, A Lesiak, PA Philipsen. Optimal sunscreen use, during a sun-holiday with a very high UV index, allows vitamin D synthesis without sunburn. Br J Dermatol. *In press*

> IL227. Invited Lecture
Symposium ENV-3 Vitamin D (Mark Farrar)

CONTRIBUTION OF NUTRITION SCIENCE TO THE VITAMIN D FIELD

Authors: Kevin Cashman[1]
Presenting Author: Kevin Cashman
1) University College Cork

Recent opinion pieces have questioned whether nutrition science is fit for purpose, suggesting that the evidence-base for dietary recommendations is populated with poor quality science and unresolved controversy. Nutrition science is accused of not keeping up with the times and making little real-world progress to stem the growing global malnutrition crisis, by failing to apply cutting-edge techniques to nutrition problems. Nutritional epidemiology has been blamed for harming public health nutrition and the public perception of science itself, by selectively reporting biased, confounded data. There is a serious public health problem of low vitamin D status. Given that the field of vitamin D has experienced an exponential increase in peer-reviewed publications over the last 50 years, it seems timely that we take these cues to reflect upon whether the expanded body of scientific literature has contributed to a deeper knowledge of vitamin D in health and disease, leading to improved nutrition policy and patient care, or whether it has led to so much confusion and controversy that progress has been impeded. This presentation will consider whether the accusations of poor science and biased reporting levelled at nutrition science are evident within the vitamin D nutrition research area and whether they have compromised dietary recommendations for vitamin D. In evaluating whether reformation is required, the presentation will discuss the confusion and controversy within the fieldand signpost common ground within the vitamin D community. It will outline vitamin D nutrition research that has presented strategies for vitamin D deficiency prevention within the population, particularly using food first approaches that could extend beyond high income settings to low- and middle-income countries. It will also outline some actions that would drive real-world progress.

> IL228. Invited Lecture
Symposium ENV-3 Vitamin D (Mark Farrar)

PIGMENT GENES NOT SKIN PIGMENTATION AFFECT UVB-INDUCED VITAMIN D

Authors: Hans Christian Wulf[Bispe], Peter Alshede Philipsen[Bispe], Pameli Datta [Bispe]
Presenting Author: Hans Christian Wulf
1) Bispebjerg Hospital, University of Copenhagen

Introduction
Low serum 25-hydroxyvitamin D (25(OH)D) levels in dark-skinned persons is generally believed to be caused by skin pigmentation which absorbs UVB and thereby may reduce the formation of 25(OH)D. The purpose of this study was to examine the relevance of skin pigmentation for 25(OH)D formation under controlled UVR exposure circumstances including the relevance of pigment genes.

Methods
Forty subjects with a wide range in skin pigmentation were selected for participation. Their skin pigmentation was measured alongside with 13 genetic (pigment SNPs), and 9 demographic parameters were chosen. Participants underwent full-body exposure with identical UVB doses for nine weeks during which serum 25(OH)D were measured on a weekly basis to examine the importance of the parameters on 25(OH)D formation. As the study took place during winter in Denmark it was not influenced by latitude, season, sun, or clothing habits, because ambient UVB during the winter months in Denmark is negligible and has no effect on 25(OH)D synthesis.

Results and Discussion
This study revealed considerable variation in 25(OH)D increase (range 2.9 to 139 nmol/l). Both constitutive and facultative skin pigmentation separately influenced the UVB induced increase in 25(OH)D linearly. However, this influence of pigmentation was lost in the presence of separate significant pigment SNPs. Sex, height, age, and seven SNPs located in the ASIP, MTAP, MIR196A29, and Solute Carrier Family genes explained 77.4% of the observed 25(OH)D variation, based on a combined linear model.

Conclusions
This study found that pigment genes supersede measured skin pigmentation but confirmed the influence of sex, age, and height on the UVB-induced 25(OH)D increase, suggesting the need for a broader focus in the search for casual parameters for low 25(OH)D levels in darker-skinned persons.

Conflicts of interest
None.

References

Norval M, Wulf HC. Does chronic sunscreen use reduce vitamin D production to insufficient levels? Br J Dermatol. 2009; 161(4): 732-6.

Bogh MK, Schmedes AV, Phillipsen PA, Thieden E, Wulf HC. Vitamin D production after UVB exposure depends on baseline vitamin D and total cholesterol but not on skin pigmentation. J Invest Dermatol. 2010; 130(2): 546-53.

Datta P, Philipsen PA, Olsen P, Petersen B, Andersen JD, Morling N, Wulf HC. Pigment genes not skin pigmentation affect UVB-induced vitamin D. Photochem Photobiol Sci. 2019; 18(2): 448-58.

> IL229. Invited Lecture
Symposium ENV-4 Algal photobiology (Felix Figueroa)

BIOLOGICAL WEIGHTING FUNCTIONS FOR MICROALGAL RESPONSES TO ULTRAVIOLET RADIATION

Authors: Patrick Neale[1]
Presenting Author: Patrick Neale
1) Smithsonian Environmental Research Center

The strong spectral dependence of most responses to UV exposure is a key property that needs to be defined to answer many questions about the environmental photobiology of microalgae, including: What is the effect of ozone depletion? How do water transparency and mixing depth mediate UV effects? How can the results of laboratory experiments with artificial UV sources be applied to responses in situ? These questions can be addressed with an experimentally determined biological weighting function (BWF). To assess the effects of UVR in an ecological context, experimental exposures should reflect the spectral balance in the environment, i.e. that exposure to short wavelength radiation (e.g. UVB) is always accompanied by even more exposure to longer wavelength radiation, such as UVA and PAR known to be primary inducers of repair mechanisms. Therefore, methods to determine microalgal BWFs use broadband exposures in combination with long-pass cutoff filters. From these results, statistical techniques are used to infer the implied BWF. For microalgae, experimental exposures to polychromatic irradiance with varying spectral content is accomplished with the photoinhibitron. An overview will be presented of the results of using this approach to estimate BWFs for UV inhibition of microalgal photosynthesis for several marine and freshwater taxa in culture and natural assemblages, and under a range of growth conditions, to understand the interaction of UV sensitivity with growth light climate, temperature, nutrient availability and ocean acidification.

> IL230. Invited Lecture
Symposium ENV-4 Algal photobiology (Felix Figueroa)

UV PHOTOPROTECTION BY RED ALGA EXTRACTS WITH HIGH CONTENT OF POLYPHENOLS AND MYCOSPORINE LIKE AMINOACIDS

Authors: Félix L.Figueroa[1], Félix Álvarez-Gómez[1], Nathalie Korbee[1]
Presenting Author: Félix L. Figueroa
1) Malaga University

The cosmeceutic industry is interested in UV screen photoprotectors with high photo- and termostability, biodegradables and with antioxidant capacity. Mycosporine like aminoacids (MAAs), low molecular weight, water-soluble and nitrogen enriched molecules, extracted from red seaweeds, are ideal sunscreens due to, in addtion to the above characteristics, they present a strong UV absorption, energy dissipation as heat and short-lived excited state (no photoproduct formation). The red alga *Hydropuntia cornea* grown under solar radiation and high ammonium content for 35 d was used as source of UV photoprotectors. The contents of polyphenols and MAAs in water extracts were high i. e 50 and 5 mg g^{-1} dry weight of biomass, respectively . MAA productivity was 237 mg MAAs m^{-2} d^{-1} being the highest MAA productivity reported until now in the bibliography for cultivated macroalgae. The cytotoxicity of the water extracts concentrated by rotary evaporation were studied by MTT assay in two human cellular lines (HaCaT and HGF). The extracts did not show any cytotoxicity activity against human cells. Finally solar protection factor (SPF) related to the erythema, mainly an UVB response, and Solar protection factor of UVA related persistent pigment darkening expressed as UVA_{SPF} were determined by using a solar simulator. Other biological effects as photocarcinogenesis, immunosupression and formation of oxygen radicals were determined. UVA/UVB ratio, critical wavelength and the new index of photoprotection (Biological effective protection factor) of the cosmetic cream was optimal. The advantageous of biological compared to chemical UV filters both for human health and the for the marine environment is discussed.

> IL231. Invited Lecture
Symposium ENV-4 Algal photobiology (Felix Figueroa)

PHOTOREGULATORY ROLES FOR BILINS IN GREEN ALGAE

Authors: John Clark Lagarias[1]
Presenting Author: John Clark Lagarias
1) University of California, Davis

Linear tetrapyrroles (bilins) are utilized by two protein superfamilies, light-harvesting phycobiliproteins and light-sensing phytochrome photosensors. Ubiquitous in cyanobacteria, phycobiliproteins have been repeatedly lost in diverse lineages of photosynthetic eukaryotes. Phytochromes are similarly absent in many eukaryotic and cyanobacterial lineages, such as chlorophyte algae of the UTC clade (Ulvophyceae/Trebouxiophyceae/Chlorophyceae). Strikingly, phytochromes have instead duplicated into small gene families in other cases, such as land plants and kelps. In land plants, phytochromes optimize photosynthetic light capture by mediating massive reprogramming of gene expression. These observations suggest that regulatory roles for phytochromes have been rendered unnecessary in some cases but not others, perhaps due to other photoreceptors. By contrast with loss of phycobiliprotein and phytochrome genes, genes for synthesis of the reduced bilin precursors of the chromophores of phytochromes and phycobiliproteins have been universally retained in all oxygenic photosynthetic organisms. This argues that bilins play essential roles independent of known photosensors or light harvesting systems in eukaryotic algae lacking phytochromes and phycobiliproteins.

We have focused on the structure, function and biogenesis of phytochromes in the green algal lineage, using the green algal species *Mesotaenium caldariorum*, *Micromonas pusilla,* and *Chlamydomonas reinhardtii,* for comparative purposes. Using characterization of phytochrome from *Mesotaenium caldariorum* and *Micromonas pusilla*, we demonstrated that green algal phytochromes utilize the blue-shifted chromophore precursor phycocyanobilin (PCB) rather than phytochromobilin (PFB) used by land plant phytochromes. Our studies also reveal that PCB is produced by different enzymes, HY2 in streptophyte algae and PCYA in prasinophytes, and that light-dependent nuclear relocalization of phytochrome already had evolved in the earliest Viridiplantae lineage.

Our studies on *Chlamydomonas* have taken advantage of reverse genetic approaches to understand the role of bilins in a green algal species that lacks phytochromes. *Chlamydomonas* retains the complete pathway for PCB biosynthesis, having two heme oxygenases (HMOX1 and HMOX2) to catalyze the production of biliverdin IXa (BV) from heme and a single PCYA enzyme to catalyze conversion of BV to PCB. Unable to secure an insertional mutant for *CrPCYA*, we examined the role of bilins by obtaining loss-of-function mutants in genes for HMOX1 and HMOX2. Our studies on these mutants implicate bilin biosynthesis to be essential for proper regulation of a nuclear gene network involved in oxygen detoxification during dark-to-light transitions and for chlorophyll synthesis in light.

> IL232. Invited Lecture
Symposium ENV-4 Algal photobiology (Felix Figueroa)

UV PHOTOPROTECTORS IN SEAWEEDS: A BRIEF PROTEOMIC INSIGHT

Authors: Fungyi Chow[1]
Presenting Author: Fungyi Chow
1) University of Sao Paulo

During the ages of early ancient Earth, between 2.5 billion and 542 million years ago, protection against harmful radiation was essential for the survival of the species and the evolution of new defenses to prevent UV-induced damage a mandatory condition. It is presume that ancestral origin of UV-protecting molecules derives from others with physiological roles, which evolved for radiation screening function. For organisms exposed to sunlight, mechanisms for reducing UV damage include: (a) physical or chemical barriers with UV-absorbing or refracting compounds as screening mechanisms; (2) non-enzymatic (carotenoids, glutathione, phenolic compounds, mycosporine like-amino acids/MAAs) and enzymatic antioxidants (SOD, CAT, glutathione peroxidase) as quenching mechanisms; and (3) repair frame to deal with UV-induced damage on DNA, proteins and lipids. The most studied photoprotective response in seaweeds is the production or accumulation of UV-absorbing compounds, including phlorotannins characteristic of brown algae and MAAs common in red algae. In its turn, insight about the functions of genes, transcripts, proteins, metabolites, comprising its interactions, is an essential approach for understanding the biological bases that determine the main physiognomies overcoming to the dynamic responses of stress-responsive mechanisms. Considering a proteomic approach, we selected the brown alga *Sargassum filipendula* as biological model to study the quantitative profile of proteins expression submitted to UVR treatments. From 767 proteins identified, 34 showed differences between the treatments, related to energy metabolism (18%), photosynthesis (18%), carbohydrate metabolism (15%), transport and catabolism (6%), ROS scavenging defense and stress related (3%), and genetic information processing (3%). The production of UV protectors and the regulatory mechanisms involved are important insight for basic and applied phycology, let us significant understanding of the mechanism undelaying stress tolerance.

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> IL233. Invited Lecture
Symposium ENV-4 Algal photobiology (Felix Figueroa)

THE USE OF MYCOSPORINE-LIKE AMINO ACIDS FOR SKIN PHOTOPROTECTION

Authors: Karl Lawrence[1], Ranko Gacesa[2], Paul Long[2], Antony Young[1]
Presenting Author: Karl Lawrence
1) St. John's Institute of Dermatology, King's College London, London, UK 2) Institute of Pharmaceutical Science, King's College London, London, UK

Topical sunscreens enhance human health by reducing sunburn and skin cancer. However, there is growing concern with their use as there is evidence now that synthetic organic filters can damage the environment and possibly be harmful to humans, sufficient that 8 out of the 16 commonly used UV filters currently licensed for use in the EU are now listed in the Community Rolling Action Plan (CoRAP) of the European Chemical Agency (ECHA) for safety evaluation. This has rekindled the search for safe biocompatible sunscreens. Mycosporine-like amino acids (MAAs) are a family of >20 secondary metabolites commonly produced by marine algae and seaweeds that reside in shallow-water environments, which are typically exposed to high levels of solar radiation. By virtue of dietary accumulation from the marine food chain, MAAs are also found in the tissues of some marine vertebrates, such as fish.

We demonstrate evidence that MAA are highly effective in inhibiting a range of UVR induced damage in a human skin model. Endpoints measured include DNA damage, oxidative stress and gene expression changes associated with photoageing, inflammation and oxidative stress. We also show MAA to have several antioxidant properties, acting as chemical quenchers and biological antioxidants by activating the cytoprotective Nrf2 pathway. This work suggests that MAA may be developed as multifunctional photoprotective compounds, acting as photostable, biocompatible UV filters with potent anti-oxidant properties.

> IL234. Invited Lecture
Symposium ENV-4 Algal photobiology (Felix Figueroa)

USE OF LIGHT EMITTING DIODES (LEDS) FOR THE INDUSTRIAL PRODUCTION OF MICROALGAE

Authors: João Varela[CCMAR], Hugo Pereira[CCMAR], Peter Schulze[CCMAR]
Presenting Author: João Varela
1) CCMAR - University of Algarve

Light emitting diodes (LEDs) are becoming one of the most prominent lighting system worldwide. By 2020, it is expected that two thirds of the global market of luminaries in terms of value will be dominated by LEDs. This increase is due to the longer durability and lower power consumption of LED luminaries (Schulze et al., 2014). Although microalgae can be grown outdoors with sunlight, in some particular cases, microalgae can benefit from the use of LED lighting, such as decreased biomass losses during the night, increased productivity at locations where sunlight is lacking during winter (high latitude countries) or in highly concentrated microalgal cultures, or for producing specific metabolites (Schulze et al., 2014, 2016). The higher costs of artificial lighting could be offset by the use of renewable energy sources, by the production of high-value compounds (e.g., astaxanthin), and better control of biomass production and quality. However, the exact combination of LEDs that should be used depends on the biology (light-harvesting pigments and regulatory photoreceptors) and the evolutionary history of the microalga to be produced (Schulze et al., 2014). Here, we will present and discuss the latest data concerning the proper use of LEDs for the production of microalgae with different evolutionary histories, focusing on chlorophytes and chromalveolates, although other groups of microalgae will also be mentioned. The possible use of flashing LEDs to improve the efficiency of the process will also be discussed (Schulze et al., 2017).

Acknowledgements
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References

Schulze, P.; Barreira, L.A.; Pereira, H.G.C.; Perales, J.A.; Varela, J.C.S. (2014). Light emitting diodes (LEDs) applied to microalgal production. *Trends Biotechnol.*, 32, 422-430.

Schulze, P.S.C.; Pereira, H.G.C.; Santos, T.F.C.; Schüler, L.; Guerra, R.; Barreira, L.A.; Perales, J.A.; Varela, J.C.S. (2016) Effect of light quality supplied by light emitting diodes (LEDs) on growth and biochemical profiles of *Nannochloropsis oculata* and *Tetraselmis chui*. *Algal Res.*, 16, 387-398.

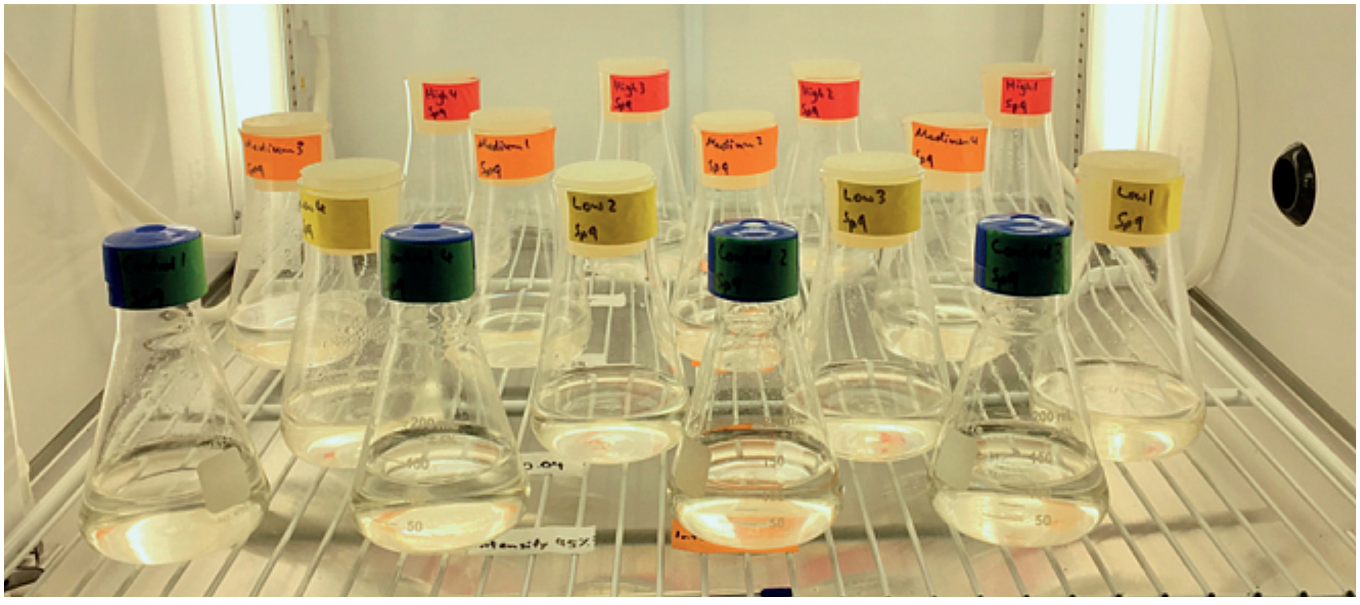
Schulze, P.S.C., Guerra, R., Schüler, L.M., Varela, J.C.S. (2017). Flashing LEDs for microalgal production. *Trends Biotechnol.*, 35, 1088-1101.

> OC102. Oral Communication
Symposium ENV-4 Algal photobiology (Felix Figueroa)

IMPACTS OF UV-B RADIATION ON THE VIABILITY AND PHYSIOLOGY OF RED SEA PHYTOPLANKTON

Authors: Sebastian Overmans[1], Susana Agustí[1]
Presenting Author: Sebastian Overmans
1) King Abdullah University of Science and Technology

Prolonged exposure to UV-B radiation is known to have a broad range of detrimental effects on living organisms. In aquatic environments, the degree of exposure is determined by both the incident radiation, as well as the transparency of the water body. One example of an extreme light environment is the Red Sea, which receives intense solar UV-B radiation all year round because of its low-latitude location. At the same time, it has highly transparent waters due to minimal concentrations of both phytoplankton and dissolved organic matter in the water column. In the present study, we aimed to assess the susceptibility to UV-B radiation of various phytoplankton taxa native to the Red Sea. Specifically, we exposed populations of 10 different phytoplankton species (five diatoms, three flagellates, two cyanobacteria) in culture to different UV-B (280–320 nm) doses in temperature-controlled indoor incubators. To evaluate UV-B impacts, we quantified mortality rates and changes in the photosynthetic efficiency of the phytoplankton populations. We identified substantial differences in the sensitivity to UV-B between the plankton species investigated, and hypothesize that UV-B is a significant abiotic stressor in the region, actively driving the latitudinal and depth distribution of phytoplankton species in the Red Sea.



> OC103. Oral Communication
Symposium ENV-4 Algal photobiology (Felix Figueroa)

STRUCTURAL LIGHT MANIPULATION FOR PHOTOSYNTHESIS IN DIATOMS

Authors: Johannes Wilhelm Goessling[1], Martin Lopez-Garcia[1]
Presenting Author: Johannes Wilhelm Goessling
1) International Iberian Nanotechnology Laboratory

Introduction

Recent studies demonstrated that natural photonic structures can modulate photosynthetic efficiencies in some plants and algae[1,2,3]. Here we present an experimental investigation of the photonic properties of the porous silicate skeleton of diatom microalgae, an extracellular matrix known as the frustule. The diatom frustule is perforated with nanometer pores and chambers allowing for chemical communication between cell and environment. These structures are arranged in strict periodicity and can thereby interact with electromagnetic radiation of sunlight.

We studied the frustule optical properties of the centric diatom *Coscinodiscus granii* with numerical analysis and Fourier image spectroscopy, which allowed for wavelength and angle resolved scattering measurement at the microscale.

Results and Discussion

The frustule of *C. granii* harbors three highly ordered 2D hexagonal lattice structures, *i.e.*, small pores on the outside, over hexagonal chambers, on cylindrical pores at this inside. Such structures remind of artificial photonic crystals and suggest similar photonic properties. We found that under particular illumination conditions these structures function like a selective light coupler for wavelengths that are more productive for photosynthesis[3]. Frustule structures also facilitate wave-guiding and redistribution of light inside the cell. Our preliminary measurements by micro-PAM techniques suggest that the photonic environment might modify the photosynthetic quantum yield under different excitation wavelengths. We speculate that the marveling colors of frustules, as observed with optical microscopes since centuries, are visual side-effects of the photonic environment for optimized light capture and photosynthesis.

Conclusion

We show that the diatom frustule couples with more productive wavelengths of light under particular illumination configuration. The unique photonic properties of the complex diatom frustule might have evolved to stimulate photosynthesis in aquatic environments, but could in future also be used as blue prints for improved industrial light harvesting processes.

Acknowledgements

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Conflicts of Interest

The authors declare that they have no conflict of interest.

References

[1] M. Jacobs, M. Lopez-Garcia, O.-P. Phrathep, T. Lawson, R. Oulton, and H. M. Whitney, *"Photonic multilayer structure of Begonia chloroplasts enhances photosynthetic efficiency"* Nat. Plants **2**, 16162 (2016).
[2] M. Lopez-Garcia, N. Masters, H. E. O'Brien, J. Lennon, G. Atkinson, M. J. Cryan, R. Oulton, and H. M. Whitney, "Light induced dynamic structural color by intracellular 3D photonic crystals in brown algae" *Science Advances. 4(April),* eaan8917 (2018).
[3] J. W. Goessling, Y. Su, P. Cartaxana, C. Maibohm, L. F. Rickelt, E. C. L. Trampe, S. L. Walby, D. Wangpraseurt, X. Wu, M. Ellegaard, and K. Michael, "Structure-based optics of centric diatom frustules : modulation of the *in vivo* light field for efficient diatom photosynthesis," *New Phytol.* (219), 122–134 (2018).

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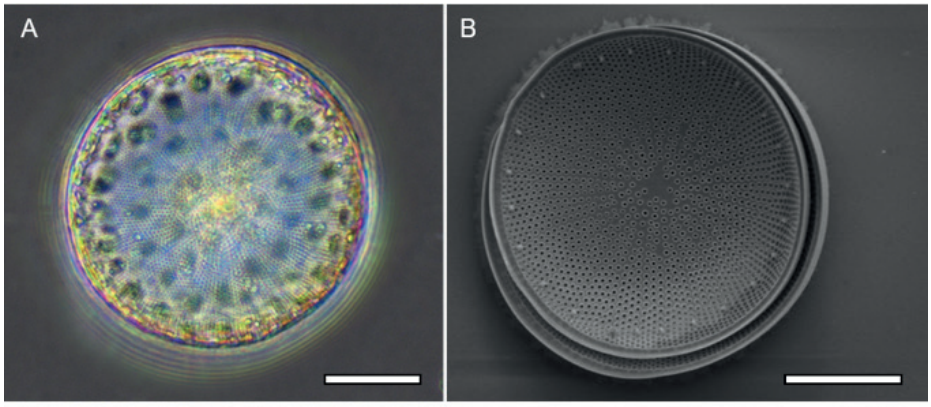

The silicate exoskeleton named the frustules surrounding diatoms. A) Life specimen in dark field showing scattering of more productive blue light. B) Oxidized frustule in Scanning Electron Microscopy. Scale bar = 20 µm

> OC104. Oral Communication

Symposium ENV-4 Algal photobiology (Felix Figueroa)

PHOTOMORPHOGENESIS OF CYANOBIUM GRACILE. PERMANENT EXCITONIC DECOUPLING OF LIGHT-HARVESTING PHYCOBILISOMES FROM PHOTOSYNTHETIC REACTION CENTERS IN RED-LIGHT GROWN CELLS

Authors: Gábor Bernát[1, 2], Tomás Zavřel[3], Eva Kotabová[2], László Kovács[4], Gábor Steinbach[5], Lajos Vörös[1], Ondřej Prásil[2], Boglárka Somogyi[1], Viktor Tóth[1]
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Photomorphogenesis is a process by which photosynthetic organisms perceive external light parameters, including light quality (color), and adjust cellular metabolism, growth rates and other parameters, in order to survive in a changing light environment. In this study we comprehensively explored the light color acclimation of *Cyanobium gracile*, a common cyanobacterium in turbid freshwater shallow lakes, using nine different monochromatic growth lights covering the whole visible spectrum from 435 nm to 687 nm. According to incident light, *C. gracile* performed great plasticity in terms of pigment composition, antenna size, and photosystem (PS) stoichiometry, to optimize their photosynthetic performance and to redox poise their intersystem electron transport chain. In spite of such compensatory strategies, *C. gracile*, like other cyanobacteria, uses blue and near far-red lights less efficiently, which involves moderate growth rates and reduced cell volume. Increased wavelength of the growth light is accompanied by increasing PS II to PS I ratios. Under unfavorable light conditions, i.e. between 500-600 nm and above 660 nm, where neither chlorophyll nor phycobilisomes absorb light sufficiently, further compensation included enhanced antenna size and/or carotenoid levels. This finding indicates a dual light-harvesting/photoprotective role of carotenoids under critical light conditions. Increased PS II to PS I ratios, which allow better light utilization in the red spectral region, is surprisingly accompanied by a partial excitonic antenna decoupling, which was the highest in the cells grown under 687 nm light. So far, similar phenomenon was known to be induced only by strong light; here we demonstrate, that such decoupling could also be induced by weak near far-red light. This suggests that suboptimal photosynthetic performance of *C. gracile* grown under near far-red light is due to a solid redox- and/or signal-imbalance, which leads to the activation of this short-term light acclimation process.

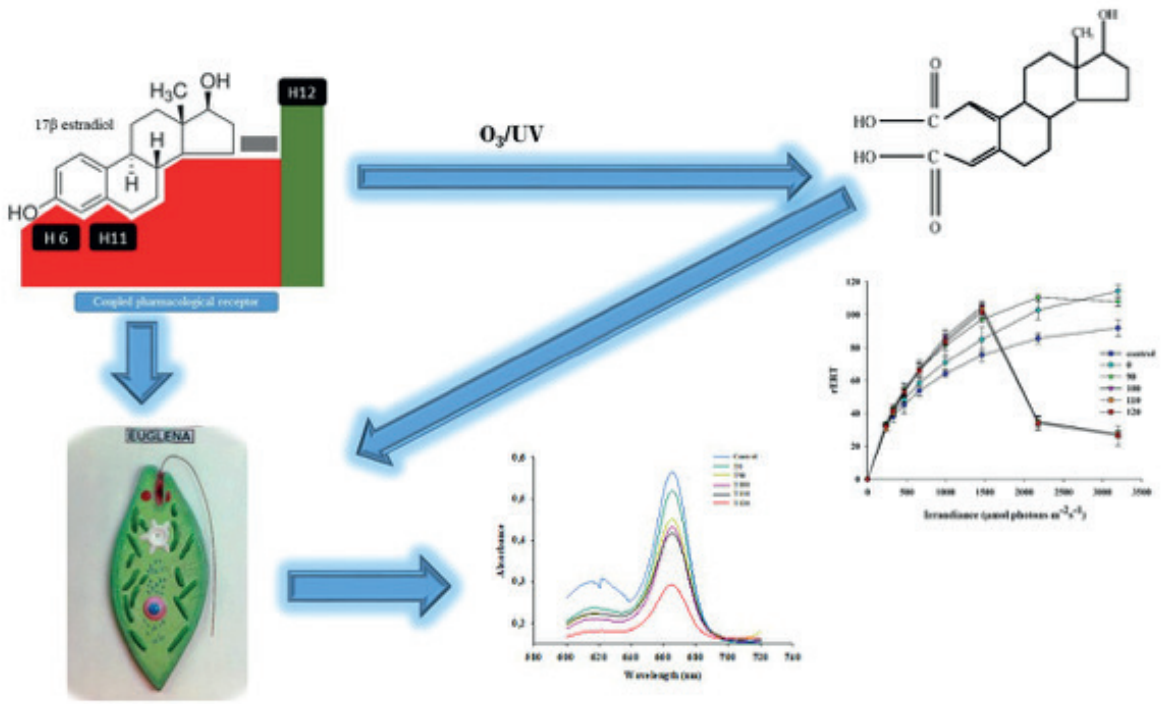
> OC105. Oral Communication

Symposium ENV-4 Algal photobiology (Felix Figueroa)

ALTERATION OF THE PHOTOSYNTHETIC ACTIVITY OF EUGLENA GRACILIS ALGAE BY RESIDUES OF 17β-ESTRADIOL HORMONES AFTER EXPOSURE TO O3 / UV

Authors: Gilmar Sidnei Eringer[1], Luciano Henrique Pinto[1], Aline Scheller[1], Julia Carolina Soares[1], Cláudia Hack Guns Correia[1], Donat-Peter Häder[2]
Presenting Author: Gilmar Erzinger
1) Graduate Program in Health and Environment - University of Joinville Region, SC, Brazil 2) Emeritus from Friedrich-Alexander University, Department Biology, Erlangen-Nürnberg, Germany

The environment is vulnerable to contaminations caused by man-made synthetic chemicals. An example of such contaminants is 17β-estradiol, a hormone that causes substantial damage and behavioral changes in animals, such as fish. The objective of this work was to evaluate the impact of the residues of this hormone obtained after an extensive oxidation process by O_3/UV in order to remove the monophenolic group and to quantify the environmental risk by measuring the photosynthetic activity of the unicellular flagellate *Euglena gracilis* upon exposure. The results demonstrate that the hydroxyls were removed but intermediates were formed, which were investigated and identified as dicarboxylic acids, compounds that destroy chlorophyll. Therefore, removal via oxidative processes using O_3/UV was not sufficient to guarantee the safety of the environment, since the algae showed a decrease in the overall photosynthetic efficiency and chlorophyll concentration caused by the presence of dicarboxylic acids. Thus, while the advanced oxidative processes eliminated the estradiol, the dicarboxylic acid compounds resulting from this removal caused physiological and behavioral alterations in the studied microorganisms.



> OC106. Oral Communication
Symposium ENV-4 Algal photobiology (Felix Figueroa)

PHOTONIC PROPERTIES OF CYANOBACTERIAL CELLS

Authors: Helder Carmen[1], Arjen Barder[2], Hugo Sinclair[3,4], Alan Lowe[3, 4], Conrad Mullineaux[1]
Presenting Author: Helder Carmen
1) School of Biological and Chemical Sciences, Queen Mary University of London 2) Department of Agrotechnology & Food Sciences, University of Wageningen 3) Institute for Structural and Molecular Biology, University College London and Birkbeck College London 4) London Centre for Nanotechnology, London

Introduction/Background
Phototaxis requires directional light perception, and recently it has been showed that individual cells of the spherical cyanobacterium *Synechocystis* sp. PCC6803 can accurately perceive the position of a light source due to micro-lensing: the cell focuses an image of the light source at the opposite periphery of the cell, where it is detected by photoreceptors in or close to the plasma membrane [1]. How can *Synechocystis* act as such an effective micro-lens?

Methods
To characterise the environment responsible for light guiding we employ quantitative phase imaging (QPI) to and Fluorescence Lifetime Imaging Microscopy (FLIM) to map the refractive index of *Synechocystis* cells; 3D-Finite Difference Time Domain (FDTD) simulations are used to model the lensing properties and other nanophotonic properties of *Synechocystis* cells.

Results and Discussion
The refractive index (*n*) of *Synechocystis* cells is not uniform. In the central cytoplasm, *n* ~1.4 (typical for the bacterial cytoplasm) while in the surrounding thylakoid membrane layers *n* is unusually high (reaching 1.5), probably due to the very high concentration of lipid and protein in this region. FDTD simulations show that a model *Synechocystis* cell with these properties acts a very effective microlens, even when the cell is immersed in water. In addition, use of FDTD has shown that the geometry of *Synechocystis* and its refractive properties, without regard to absorption, lend themselves to enhancing the flux of photosynthetically relevant wavelength in the region of the thylakoid membrane. This effect seems to be modulated when the absorptive properties of the thylakoid region is taken into account.

Conclusions
Directional light perception in *Synechocystis* is enabled by the specific optical properties of the cell, which allow it to act as a robust micro-lens. The implication is that these optical properties enable the cell to differentially respond to a range of wavelengths.

Conflicts of Interest
No Conflicts of Interest

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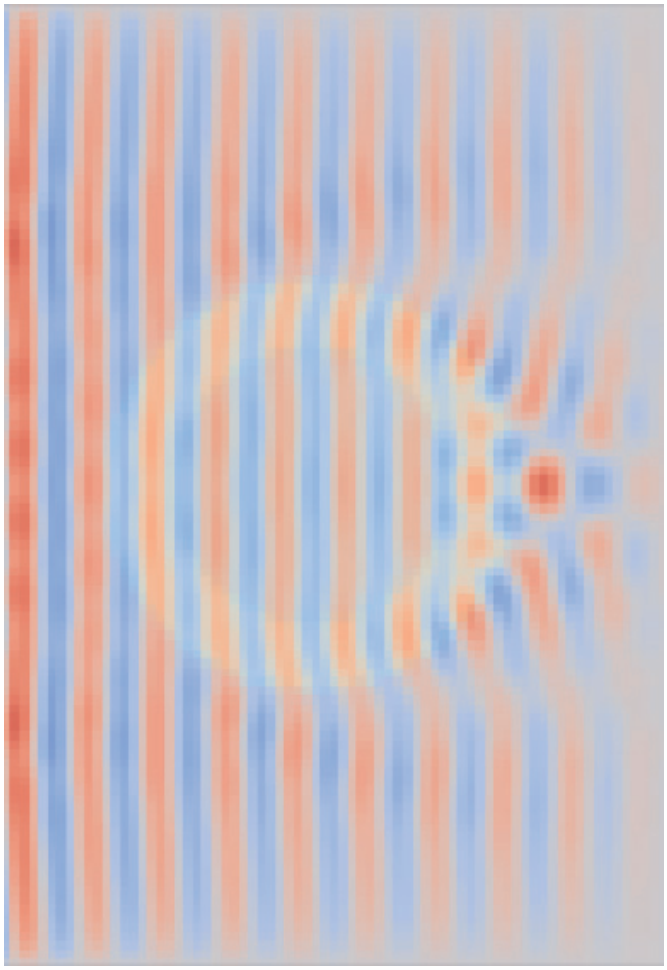

Incident plane wave light from the left interacting with a model cell. *685nm plane wave light interacts with a model representing Synechocystis (3 micrometre in dimeter). The model consists of a shell and core model, with the core repressing cytoplasm (refractive index 1.4) and the shell representing thylakoid (refractive index 1.5). Focusing of the light can be seen on the right of the cell.*

> OC107. Oral Communication

Symposium ENV-4 Algal photobiology (Felix Figueroa)

ECOPHYSIOLOGICAL RESPONSES UNDER INCREASED TEMPERATURES MEDIATED BY FUTURE CLIMATE CHANGE SCENARIOS ON SPECIES O INTERTIDAL MACROALGAE

Authors: Paula S.M. Celis-Plá[1], Fernanda Rodríguez-Rojas[1], Félix L. Figueroa[1], Fabiola Moenne[1], Murray Brown[2], Nelso Navarro[3], Iván Gómez[4], Claudio Sáez[1]
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Recent findings have demonstrated that Antarctica is warming up at one of the world´s highest rates. Macroalgae are base of trophic networks in coastal rocky shores from inter-tropical to polar latitudes, thus, their diversity and abundance control the complexity of entire coastal ecosystems. Seaweeds from polar regions have to thrive with extreme environmental conditions, such as increased UV exposure, low light and fluctuating temperatures; even though, these organisms are dominant in benthic and intertidal Polar ecosystems. In this context, we performed a simple experiment to determine if fluctuations in water temperature affected physiological parameters in terms of photosynthetic activity in three species of intertidal macroalgae: *Adenocystis utricularis* (brown), *Pyropia endivifolia* (red) and *Monostroma hariotii* (green). Samples were collected in Punta Artigas (King George Island, Antarctica), and acclimated at 2°C in the laboratory with filtrated seawater. In parallel, other samples were subject to increased temperatures of 8 °C for five days; these temperatures are predicted in negative scenarios considering predictions of climate change by the end of the XXI Century. The evaluation of photosynthetic activity as maximal quantum yield (F_v/F_m) as photoinhibition capacity, the maximal electron transport rate (ETR_{max}) as estimator of photosynthetic production, and non-photochemical quenching (NPQ_{max}) as photoprotection capacity in algae collected and exposure for 5 days in control (2°C) and higher temperature (8°C) treatments, were evaluated at the beginning of experiments and after four days. The photosynthetic activity showed that elevated temperature levels benefitted these macroalgae, although their responses varied depending on ambient temperature, thus in *A. utricularis* under elevated temperature had the highest ETR_{max}, F_v/F_m and NPQ_{max} whereas in *P. endivifolia* the ETR_{max} from control and elevated temperature conditions were lower, respect to the others macroalgae. Results demonstrated differential responses between the macroalgae species assessed, which put in evidence interspecific bio-optical characteristics, photoinhibition, photosynthetic and photoprotective capacities.

Acknowledgements
Inach project RG_10_18 and UPA-CEA project 1795, granted to Paula S.M. Celis-Plá, and Inach project RT_09-16, granted to Claudio A. Sáez.

> P113. Poster

Symposium ENV-4 Algal photobiology (Felix Figueroa)

PROSPECTING PHOTOPROTECTIVE CAPACITIES IN MARINE MACROALGAE IN SOUTHERN SPAIN

Authors: José Bonomi Barufi[1], Geniane Schneider[1], Nathalie Korbee[2], Félix Álvarez-Gómez[2], Patricia Chaves[2], Julia Vega[2], Félix L. Figueroa[2]
Presenting Author:José Bonomi Barufi
1) Federal University of Santa Catarina 2) Málaga University

Searching for new sources of photoprotective compounds is a fundamental aspect to offer new biotechnological alternatives to cosmeceutical industry. Marine macroalgae have shown many potentialities, with some known substances already being used to this perspective, such as mycosporine-like amino acids (MAAs)(1), but new alternatives must be taken into account, including properties like radiation avoidance and antioxidant capacity, among others (2). Then, 23 algal and 1 lichen samples were collected in three areas in Southern Spain: La Araña (Málaga), Tarifa (Cádiz) and samples from indoor-cultivation conditions in a greenhouse located in Málaga. Five Chlorophyta, four Ochrophyta and 13 Rhodophyta were analyzed. Hydro-ethanolic extracts (ethanol: distilled water, 1:1) were obtained from frozen material, and were utilized for determination of UV-Vis absorption spectra, total phenolic compounds, antioxidant activity (ABTS), MAAs, solar and UVA- protection factors (SPF and UVAPF). *Porphyra umbilicalis* and *Pyropia elongata* presented the highest absorbance values at 330 nm, while *Ulva fasciata* showed a prominent peak at 290 nm. In the visible spectra, fucoxanthin peak was strongly evident in the brown algal species, while green algae presented characteristic chlorophyll *a* and *b* peaks at 447 nm, 620 and 664 nm. Polyphenols and ABTS activity were much higher in *Cystoseira tamariscifolia*, *Sargassum vulgare*, and *Lichina pygmaea* in comparison to the other species. MAAs were found mainly in the red algal species, and species of Bangiales showed the highest amounts. SPF close to 50 and UVAPF of 12 were found with *S. vulgare* tissues. In general, protection factors increase with algal tissue concentrations. Our results point out to *S. vulgare* and *P. umbilicalis* as the main potential sources of antioxidant and phtoprotective compounds and further tests in sunscreens formulae will be conducted in the future. Biomass availability (algal abundance and cultivation facilities) for the studies also should be taken into account.

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The authors declare no conflicts of interest.

References

1. Navarro NP et al. 2018. Mycosporine-like amino acids form red algae to develop natural UV sunscreens. In: Sunscreens: source, formulation, efficacy and recommendations Edited by R.P. Rastogi, 99-129 pp. Biochemistry Research Trends : Nova Science Publishers Inc.ISBN 978-1-53613-294-6.
2. Álvarez-Gómez F et al. 2019. UV Photoprotection, citotoxicity and immulogicalcapacity of red alga extracts. Molecules 24, 341; doi:10.3390/molecules24020341

> P114. Poster

Symposium ENV-4 Algal photobiology (Felix Figueroa)

CATALYTIC NANOCOMPOSITES FROM DIATOMS MICROALGAE

Authors: Danilo Vona[1], Roberta Ragni[1], Stefania Roberta Cicco[2], Gabriella Leone[1], Marco Lo Presti[1], Gianluca Maria Farinola[1]
Presenting Author: Danilo Vona
1) Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, Via Orabona 4, 70126, Bari, Italy 2) Istituto per la chimica dei composti organometallici (CNR-ICCOM)-Bari, Bari 70126, Italy

Many catalysts such as transition metal complexes, metal nanoparticles and enzymes, suffer from low stability, low shelf-life and reduced recyclability in operational conditions. Immobilization of the catalytic species onto solid support is used to overcome such issues. Silica has been widely used since it is chemically stable, biocompatible, highly hydrophilic and optically transparent. Moreover, the diffusion of reagents through the porous material balances the partial loss of catalyst activity due to the rigid confinement. In the specific case of enzymes, silica protection can prevent protein denaturation. Most protocols for the production of mesoporous silica-based materials require toxic silicon alkoxides, high temperature and pressure, leading to high consumption of energy. An environmentally friendly alternative to synthetic mesoporous silica is provided by diatoms microalgae that generate highly porous silica shells called frustules. Frustules biosilica is the result of a metabolic biomineralization process of inorganic silicon salts occurring in these photosynthetic microorganisms. Diatom frustules display high surface area, tunability of pore size and biocompatibility.[1] In this work, living *Thalassiosira weissflogii* diatoms and their extracted biosilica shells have been decorated with enzymes (lipase, laccase, tyrosinase), palladium and silver [2] nanoparticles, and transition metal complex [3] to produce nano-biohybrid systems for ecosustainable catalysis and bioremediation. All the resulting materials were characterized by spectroscopy and microscopy.

These diatoms biosilica-based nanomaterials were demonstrated to be efficient solid supports for enzymes and catalytically active metals. The immobilization of these species on both living diatoms and extracted biosilica shells was successfully carried out working in eco-friendly conditions. In particular, for enzymes supported on living cells, kinetic parameters, recycle ability and enzymatic activity were investigated over culture time. The new hybrid materials obtained pave the way to low cost, green and time earning catalysis application.[4]

Acknowledgements

This work was supported by the European Commission through the EU project 800926-HyPhOE (Hybrid Electronics based on Photosynthetic Organisms).

References

1 R. Ragni, S. R. Cicco, D. Vona, G. M. Farinola, Adv. Mater. 30(19), No. 1704289 (2018)
2 D. Vona, S. R. Cicco, R. Ragni, G. Leone, M. Lo Presti, G. M. Farinola, MRS Commun. 8(3), 911−917 (2018)
3 G. Della Rosa, D. Vona, A. Aloisi, R. Ragni, R. Di Corato, M. Lo Presti, S.R. Cicco, E. Altamura, A. Taurino, M .Catalano, G.M. Farinola, ACS Sustain Chem Eng, 7 (2), 2207–2215 (2019)
4 R. Ragni, S. R. Cicco, D. Vona, G. M. Farinola, Adv. Mater. 30(19), No. 1704289 (2018)

> P115. Poster

Symposium ENV-4 Algal photobiology (Felix Figueroa)

PHYSIOLOGICAL ROLE OF NAD KINASE IN CYANOBACTERIUM SYNECHOCYSTIS SP. PCC 6803

Authors: Yuuma Ishikawa[1], Kintake Sonoike[2], Hihara Yukako[1], Maki Kawai-Yamada[1]
Presenting Author: Yuuma Ishikawa
1) Saitama University 2) Waseda University

NAD^+ and $NADP^+$ (oxidized forms), or NADH and NADPH (reduced forms) act as oxidizing agents or reducing agents in electron-transfer steps in several metabolic pathways. Phosphorylation of NAD(H) to NADP(H) is performed by the enzyme NAD kinase (NADK). Based on BLAST searches of the cyanobacterial genomes available in the NCBI GenBank database, it was reported that almost all cyanobacteria possess two types of NADKs, despite the apparent lack of subcellular compartments within these cells. Consistent with these BLAST analyses, *Synechocystis* sp. PCC 6803 also harbors two NADK-encoding genes (*sll1415* and *slr0400*). However, it is not apparent why cyanobacteria, which have simple (prokaryotic) cell structures, have multiple NADKs, and the role of the distinct NADK paralogues remains unclear.

When genetic mutants for *sll1415* and *slr0400* were cultured under photoheterotrophic growth conditions, only the *sll1415*-deficient cells showed a growth defect. Furthermore, we reported that the *sll1415*-deficient mutant showed a growth-impaired phenotype under photomixotrophy (with 12-h light/12-h dark cycling). On the contrary, we found that only the *slr0400* disruptant showed high light sensitivity. Based on the results of chlorophyll fluorescence measurement, *slr0400* is essential for the proper photosynthetic machinery of PS II. Furthermore, the rate of electron transport from Q_A to Q_B, which was indicated by the initial rate of fluorescence decay after the flash, was impaired in *slr0400*-deficient mutant compared to WT. However, *slr0400*-deficient mutant did not show any difference in the kinetics of light-induced NADPH formation compared with the WT. These results suggest that the defect is in PS II rather than $NADP^+$ supply to the PS I.

Furthermore, we recently found that *slr0400*-deficient mutant showed a fast-growth phenotype under photomixotrophy (with 12-h light/12-h dark cycling). Based on the determination of NAD(P)(H) content, *slr0400*-deficient mutant accumulated NAD^+ under photoautotrophic condition compared with the WT. Therefore, we speculate that *slr0400* may have a key role in suppression of the heterotrophic metabolism in *Synechocystis* sp. PCC 6803.

References

Ishikawa et al., 2016. Journal of Plant Physiology, 205: 105-112.
Ishikawa et al., 2019. The Plant Journal, 98: 654-666.

> IL235. Invited Lecture
Symposium ENV-5 Stomatal responses to light (Alistair M. Hetherington)

METABOLOMICS OF RED LIGHT-INDUCED STOMATAL OPENING

Authors: Mengmeng Zhu[1], Sixue Chen[2], Sarah M. Assmann[1]
Presenting Author: Sarah M. Assmann
1) Penn State University 2) University of Florida

Stomata are microscopic pores in leaf surfaces that allow both carbon dioxide influx for photosynthetic carbon capture by the interior mesophyll cells of the leaf and transpirational water vapor efflux. Stomatal apertures are defined and regulated by pairs of guard cells that perceive and transduce signals relevant to both photosynthesis and plant water status. Signal perception leads to guard cell volume changes and consequent changes in stomatal aperture that alter rates of gas exchange. Both blue and red light trigger stomatal opening, thereby increasing carbon dioxide availability under conditions conducive to photosynthesis. However, there has been debate regarding the extent to which red light-induced stomatal opening arises from direct guard cell sensing of red light vs. indirect guard cell responses as a result of red light influences on mesophyll photosynthesis. Here we identify conditions that result in red light-stimulated stomatal opening in isolated epidermal peels and red light-induced swelling of isolated guard cell protoplasts, firmly establishing a direct guard cell response to red light. We then employ metabolomics workflows utilizing gas chromatography mass spectrometry (GC-MS/MS) and liquid chromatography mass spectrometry (LC-MS/MS) for metabolome profiling and identification of Arabidopsis guard cell metabolic signatures in response to red light in the absence of the mesophyll. We quantified 223 metabolites in Arabidopsis guard cells, with 104 found to be red light responsive. These red light-modulated metabolites participate in the tricarboxylic acid (TCA) cycle, carbon balance, phytohormone biosynthesis, and redox homeostasis. The red light-modulated guard cell metabolome reported here provides fundamental new information concerning autonomous red light signaling pathways in guard cells

> IL236. Invited Lecture
Symposium ENV-5 Stomatal responses to light (Alistair M. Hetherington)

ENVIRONMENTAL REGULATION OF STOMATAL DEVELOPMENT

Authors: Stuart Casson[1], Nicholas Zoulias[1], Jim Rowe[2]
Presenting Author: Stuart Casson
1) University of Sheffield 2) Sainsbury Laboratory, University of Cambridge

Our work is focused on understanding the signalling mechanisms that mediate plant developmental changes in response to environmental signals. Stomata, the microscopic pores on the leaf surface, are an excellent model for examining how environmental signals modulate plant development. Factors such as light quantity and quality as well as atmospheric carbon dioxide have a major impact on stomatal development. Using a combination of genetic and molecular tools our work has demonstrated that plant photoreceptors, significantly phyB, play a critical role in regulating stomatal development in response to environmental signals. We will present data that examines the mechanism by which both phyB and a photoreceptor independent pathway regulates stomatal development in response to environmental signals.

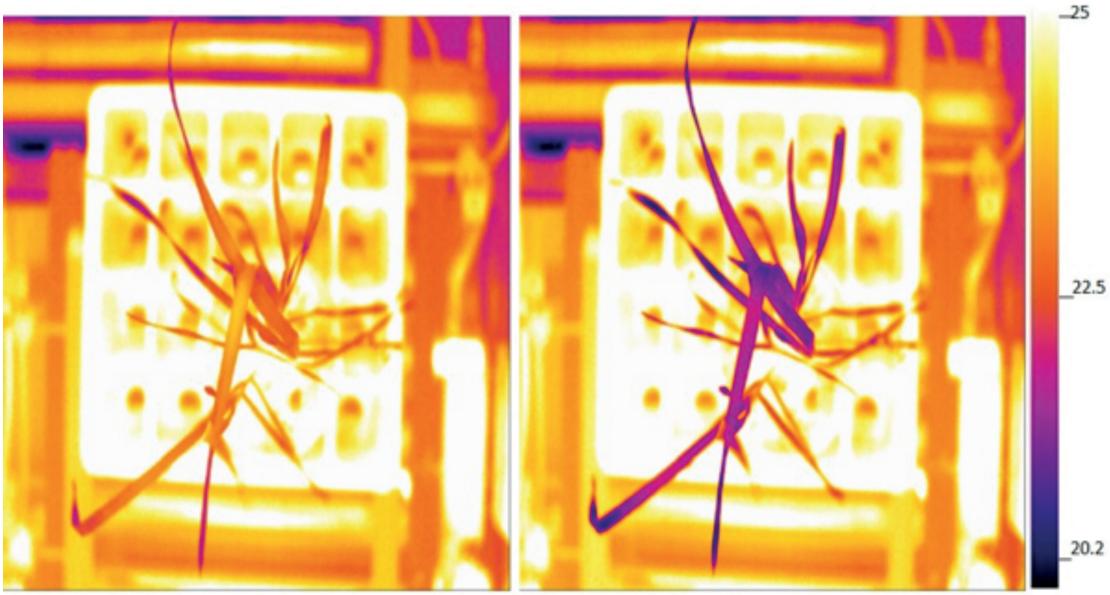
> IL237. Invited Lecture
Symposium ENV-5 Stomatal responses to light (Alistair M. Hetherington)

STOMATAL BLUE LIGHT RESPONSE: IMPACT ON ASSIMILATION AND WATER USE EFFICIENCY

Authors: Tracy Lawson[1], Jack Matthews[1], Silvere Vialet-Chabrand[1]
Presenting Author: Tracy Lawson
1) University of Essex

Stomata are gatekeepers to gaseous exchange between the atmosphere and the leaf, controlling, CO_2 uptake for photosynthesis and water loss through transpiration. Stomata adjust aperture in response to changing environmental cues to balance CO_2 uptake and water loss. In a naturally fluctuating environment, stomata and photosynthesis are continually experiencing and adjusting to a variable light intensity. Stomatal responses depend not only on the intensity of the light but also the wavelength, and two responses have been recognised. The 'red-light' response is been linked to mesophyll demands for photosynthesis, whilst the blue light response occurs at low light levels and is independent of photosynthesis. Additionally, stomatal responses are not always synchronised with mesophyll responses, as stomatal movements can be an order of magnitude slower than the more rapid photosynthetic responses. This means that under red blue combinations stomata tend to be more open than under red alone, and therefore the ratio of carbon gain to water loss through stomatal conductance (g_s), known as intrinsic water use efficiency (W_i), is reduced. We have quantified the impact of blue light on stomatal conductance g_s, assimilation rate (A) and W_i in a range of different species and have compared these responses to effect of red light. We discuss these findings in the light of manipulating the blue light response for improving W_i in crop plants.

Acknowledgements
We acknowledge BBSRC (grant no: BB/S005080/1 awarded to TL) for funding this work.



> IL238. Invited Lecture
Symposium ENV-5 Stomatal responses to light (Alistair M. Hetherington)

STOMATAL RESPONSES TO UV-A

Authors: Alistair Hetherington[Unive]
Presenting Author: Alistair Hetherington
1) University of Bristol

Although UV-A radiation (315-400 nm) represents 95% of the UV radiation reaching the earth's surface, surprisingly little is known about its effects on plants. Recent unpublished work from our lab shows that UV-A inhibits the opening of Arabidopsis stomata and this requires a reduction in the cytosolic level of cGMP. This process is independent of UVR8, the UV-B receptor. We found that a cGMP activated phosphodiesterase was responsible for the UV-A-induced decrease in cGMP in Arabidopsis. The phosphodiesterase gene has been lost from the genomes of metazoans but are otherwise conserved as single copy genes across the tree of life. In longer term experiments UV-A radiation increased growth and decreased water use efficiency. These results will be discussed during the lecture.

> IL239. Invited Lecture
Symposium ENV-5 Stomatal responses to light (Alistair M. Hetherington)

UNCOUPLING DAYTIME AND NIGHTTIME STOMATAL DYNAMICS

Authors: Florent Pantin[1]
Presenting Author: Florent Pantin
1) Montpellier SupAgro, UMR LEPSE

Every night, most plants lose a substantial amount of water through their stomata that remain partly open. This nocturnal transpiration potentially results in lower water use efficiency (WUE) and enhanced probability of drought occurrence. Breeding for drought-tolerant plants by reducing night-time water loss therefore appears as an appealing strategy. However, low transpiration at night may also correlate with lower photosynthesis if stomata are constitutively closed or sparse. Little is known about the mechanisms that specifically control the magnitude or dynamics of nocturnal transpiration. To address this question, we use low- and high-throughput methods (gas exchange, gravimetry on detached leaves or whole plants) to explore the diel (24-h) transpiration of Arabidopsis and grapevine in controlled conditions. We analyse Arabidopsis mutants to test specific hypotheses on the control of nighttime stomatal dynamics and its mechanistic relationship with daytime processes. We are also phenotyping a grapevine diversity panel to perform a genome-wide association study (GWAS) in order to identify loci that uncouple daytime from nighttime transpiration. Disrupting this relationship may enhance WUE in plants.

> IL240. Invited Lecture

Symposium ENV-5 Stomatal responses to light (Alistair M. Hetherington)

OPTOGENETIC MANIPULATION OF STOMATAL KINETICS IMPROVES PLANT GROWTH AND WATER USE EFFICIENCY

Authors: Maria Papanatsiou[1], Jan Petersen[1], Mike Blatt[1], John Christie[1]
Presenting Author: John Christie
1) University of Glasgow

Stomata serve dual and often conflicting roles, facilitating carbon dioxide influx into the plant leaf for photosynthesis and restricting water efflux via transpiration. Strategies for reducing transpiration without incurring a cost for photosynthesis must circumvent this inherent coupling of carbon dioxide and water vapor diffusion. We expressed the synthetic, light-gated K^+ channel BLINK1 in guard cells surrounding stomatal pores in Arabidopsis to enhance the solute fluxes that drive stomatal aperture. BLINK1 introduced a K^+ conductance and accelerated both stomatal opening under light exposure and closing after irradiation. Integrated over the growth period, BLINK1 drove a 2.2-fold increase in biomass in fluctuating light without cost in water use by the plant. Thus, we demonstrate the potential of enhancing stomatal kinetics to improve water use efficiency without penalty in carbon fixation.

> IL273. Invited Lecture
Symposium ENV-5 Stomatal responses to light (Alistair M. Hetherington)

BLUE-LIGHT-INDUCED GUARD CELL STARCH DEGRADATION IS REQUIRED FOR FAST STOMATAL OPENING KINETICS

Authors: Diana Santelia[1]
Presenting Author: Diana Santelia
1) ETH Zürich

Starch mobilization in guard cells of *Arabidopsis thaliana* is correlated with a rapid increase in stomatal pore aperture. The activity of enzymes involved in guard cell starch breakdown is under tight control of the phototropin-mediated blue light-signaling pathway, involving the activation of a plasma membrane H^+-ATPase as a prerequisite for starch breakdown. Double mutant plants lacking the glucan hydrolyzing enzymes β-amylase 1 (BAM1) and α-amylase 3 (AMY3) show slow opening responses and reduced aperture amplitudes. It remains unknown whether impaired starch degradation is a limiting factor for solute transport and accumulation, potentially causing the retarded opening response. We report the absence of significant differences in blue light-induced H^+-pumping and K^+channel activities between wild-type and stomatal starch-degrading mutant *amy3bam1* guard cells, suggesting that metabolites derived from starch are not directly required for membrane ion transport, but rather function as counter-ions and organic osmotica. To explore the impact of guard cell starch contents on stomatal opening kinetics, gas exchange parameters and guard cell starch granule area were determined from plants exposed to a so-called “two-pulse light regime” with alternating 2h pulses of light and darkness after the end of the night. We demonstrate that during light-induced stomatal opening, fast stomatal opening kinetics precisely correlate with the rate and amount of guard cell starch degradation. Defective guard cell starch breakdown in *amy3bam1* mutant plants results in a calculated increase in the time constant for opening of 40 min. In contrast to blue light, fast stomatal opening kinetics and the amplitude of stomatal opening under red light are independent of starch degradation but depend on the import of mesophyll-derived sugars to guard cells, which requires the activity of the plasma membrane H^+-ATPase. Our findings show that fast stomatal opening kinetics to light in Arabidopsis depend on a tight correlation between membrane ion transport and metabolic fluxes.

SYMPOSIUM COMMUNICATIONS

PHOTOSENSORY BIOLOGY

> IL246. Invited Lecture
Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

DYNAMICS OF BACTERIORHODOPSIN ACTIVATION STUDIED AT SYNCHROTRONS AND X-RAY LASERS

Authors: Joerg Standfuss[1]
Presenting Author: Joerg Standfuss
1) Paul Scherrer Institut

Time-resolved serial crystallography provides exciting new opportunities to study the structural dynamics of light-sensitive proteins. By integrating sample efficient high viscosity injectors into pump probe setups, it is now possible to determine whole series of molecular structures at precise times after activation to better understand how these proteins function.

Based on our recent studies of the light-driven proton pump bacteriorhodopsin (bR), I will outline the possibilities but also the challenges that have to be overcome before we can routinely study structural rearrangements at ambient temperature and in real time. A total of 41 temporal snapshots ranging from the femtosecond to the millisecond regime allowed us to study the bR photocycle with astounding detail. Mechanistically bR can be divided into an extracellular half and a cytoplasmic half with the retinal chromophore positioned roughly in the middle of the membrane. The first principal step in the pumping mechanism is the light induced isomerization of retinal in the femtosecond range, which provides the energy for the reaction (1). In the second step, the energy is used to change the protein conformation within microseconds to allow proton release from the retinal Schiff base towards the extracellular release group via a water mediated hydrogen-bonding network (2). In the third principal step, the protein changes again after several milliseconds to allow uptake of a proton from the intracellular side of the membrane (3). These sequential rearrangements throughout the bR photocycle follow the basic predictions of an alternate access model and provides a template to understand the principal transport steps in other membrane pumps.

References

1. Nogly P, et al. (2018) Retinal isomerization in bacteriorhodopsin captured by a femtosecond X-ray laser. *Science* aat0094 :10.1126–science.aat0094.
2. Nango E, et al. (2016) A three-dimensional movie of structural changes in bacteriorhodopsin. *Science* 354(6319):1552–1557.
3. Weinert T, et al., Proton uptake mechanism in bacteriorhodopsin captured by serial synchrotron crystallography, *under evaluation*

> IL245. Invited Lecture
Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

PROTON TRANSFER REACTIONS IN RETINAL PROTEINS: FROM BACTERIORHOPSIN TO CHANNELRHODOPSIN-2

Authors: Victor A. Lorenz-Fonfria[1]
Presenting Author: Victor A. Lorenz-Fonfria
1) Institute of Molecular Science (ICMol), Universitat de Valencia, Spain

The vectorial transport of protons across membranes by proton pumps is central to cellular bioenergetics. The smaller and best understood proton-pump is bacteriorhodopsin (BR), a light-driven proton pump of 248 residues. Vectorial proton transport by BR is energized by retinal photo-isomerization and it is accomplished by a series of internal proton transfer reactions, proton release to the extracellular medium, and proton uptake from the cytoplasmic medium.[1] Proton transfer also occurs in other membrane proteins, where it can be involved in its activation and/or in its regulation mechanism, as it seems to be the case for the microbial rhodopsin channelrhodopsin-2,[2] the first identified light-gated cation channel.[3]

Fourier transform infrared (FT-IR) difference spectroscopy stands up by its sensitivity to protonation changes. Often in combination with site-directed mutagenesis, FT-IR difference spectroscopy has allowed to identify proton transfer reactions between deprotonating and protonating groups. On the other hand, proton release and uptake events have been mostly characterized by UV/vis spectroscopy using pH-sensitive dyes, as FT-IR spectroscopy has been traditionally silent to protonation changes in the medium.

In the first part of my talk I will briefly review how time-resolved FT-IR difference spectroscopy has contributed to our understanding of proton transfers in BR and ChR2, highlighting past and present controversies.

In the second part I will explain how we traced proton release and uptake events in the proton-pumping mechanism of BR by FT-IR difference spectroscopy using buffer molecules as vibrational pH-sensitive probes.[4] Briefly, we confirmed that the source of the release proton is the deprotonation of the so-called proton release complex (PRC), a complex in the extracellular domain of bacteriorhodopsin where an excess proton is shared by a cluster of internal water molecules and/or ionic E194/E204 carboxylic groups.[5] In contrast, contrary to the accepted model,[1] proton uptake occurs after reprotonaton of Asp96, which cannot be the group accepting a proton from the CP medium. We propose that Asp96 reprotonates from the proton uptake complex (PUC), a cluster with an excess proton reminiscent to the PRC but located in the cytoplasmic domain, and the PUC takes a proton back from the CP medium.

The above commented results not only call for a reevaluation of the last proton transfer steps in bacteriorhodopsin, but show the importance of resolving by the same technique both internal and external protonation changes in proteins to more accurately reveal the sequence of proton transfers.

References

(1) Lanyi, J. K. *Biochim. Biophys. Acta* **2006**, *1757* (8), 1012–1018.
(2) Fonfría, V. a; Heberle, J. *Biochim. Biophys. Acta* **2014**, *1837* (5), 626–642.
(3) Nagel, G.; Szellas, T.; Huhn, W.; Kateriya, S.; Adeishvili, N.; Berthold, P.; Ollig, D.; Hegemann, P.; Bamberg, E. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100* (24), 13940–13945.
(4) Lorenz-Fonfria, V. A.; Saita, M.; Lazarova, T.; Schlesinger, R.; Heberle, J. *Proc. Natl. Acad. Sci.* **2017**, 201707993.
(5) Wolf, S.; Freier, E.; Gerwert, K. *Biophys. J.* **2014**, *107* (1), 174–184.

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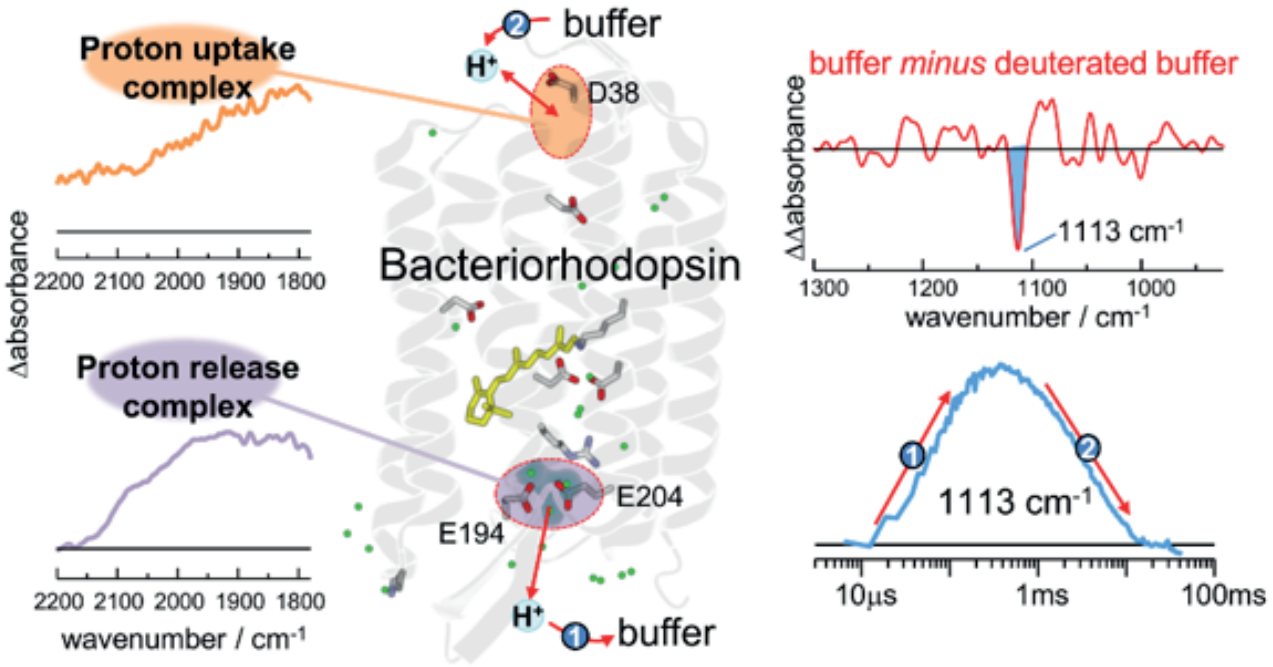
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> IL243. Invited Lecture
Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

NATURAL ANION CHANNELRHODOPSINS (ACRs): DIVERSITY AND MECHANISMS

Authors: Elena G. Govorunova[1], Oleg A. Sineshchekov[1], Hai Li[1], Christopher T. Schafer[1], John L. Spudich[1]
Presenting Author: Elena G. Govorunova
1) McGovern Medical School UTHealth

Cation-conducting channelrhodopsins (CCRs) that function as phototaxis receptors in flagellate green (chlorophyte) algae have been extensively used for optical control of cellular excitability (optogenetics). We discovered two additional channelrhodopsin families in the phylogenetically distant cryptophyte algae [1]. One of them comprises channelrhodopsins with strictly anion conductance, which we named anion channelrhodopsins (ACRs) [2]. Several ACRs generate large hyperpolarizing photocurrents in neurons by chloride conductance and have proven to be the most efficient optogenetic tools to inhibit neuronal activity and to photocontrol behavior in worms, flies, zebrafish, ferrets and mice. We have identified 35 native ACR variants highly diverse in conductance, current kinetics, and spectral sensitivity. Moreover, we have been able to further adjust some of these properties by strategically placed mutations, which allowed us to expand the range of the time domains of optogenetic neuronal silencing from a few milliseconds to tens of seconds.

Opposite to CCRs, in which photoisomerization of retinal causes rapid transfer of the proton from the retinylidene Schiff base chromophore to the protein followed by channel opening, we find that the ACR photocycles exhibit first rapid channel opening followed by very late deprotonation of the Schiff base [3]. Our X-ray crystal structure of the dark state of ACR1 from *Guillardia theta* (*Gt*ACR1) [4] revealed a novel photoactive site configuration that maintains the retinylidene Schiff base protonated when the channel is open. We observed a narrow continuous tunnel that spans the entire membrane between the extracellular to cytoplasmic surfaces of the protein which we propose is the anion conduction pathway. The tunnel shows three constrictions, one of which is the protonated Schiff base photoisomerization site. A structure of the channel open state is not yet available. Mutagenesis screening for changes in the photocurrent amplitude and voltage dependence indicates that concerted expansion of the three constricted regions around the tunnel forms the open channel. We will present results that further contribute to our understanding of ACR anion conduction mechanisms and provide clues for rational engineering of ACR molecules to increase further their optogenetic utility.

References

[1] Govorunova E.G., Sineshchekov O.A., Li H. and Spudich J.L. (2017) *Annu. Rev. Biochem.* 86: 845-872.
[2] Govorunova E.G., Sineshchekov O.A., Liu X., Janz R. and Spudich J.L. (2015) *Science* 349: 647-650.
[3] Sineshchekov O.A., Li H., Govorunova E.G., and Spudich, J.L. (2016) *PNAS USA* 113: E1993-2000.
[4] Li H., Huang, C.Y., Govorunova E.G., Schafer, C.T., Sineshchekov O.A., Wang, M., Zheng, L. and Spudich J.L. (2019) *eLife* 8: e41741.

> IL241. Invited Lecture
Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

A BOTTOM-UP APPROACH TO UTILIZING MICROBIAL RHODOPSINS FOR OPTOGENETICS

Authors: Yuki Sudo[1]
Presenting Author: Yuki Sudo
1) Okayama University

Microbial rhodopsin is a member of the seven-transmembrane photoreactive protein family that uses a retinal pigment (vitamin-A aldehyde) as a chromophore, which is covalently attached to the apoprotein opsin via a protonated Schiff base linkage with a perfectly conserved Lys residue. Visible light absorption triggers *trans-cis* photoisomerization of the retinal chromophore and induces structural changes in the protein moiety during photoreaction, resulting in a variety of biological functions. In addition to their biological significance, rhodopsin enables precise spatiotemporal control of biological activity as a genetically-encoded tool for optogenetics.

In the presentation, I would like to introduce a bottom-up approach to utilizing microbial rhodopsins for optogenetics from the viewpoints of (i) functional diversity, (ii) functional and structural analysis and (iii) development of tools for optogenetics.

References

(2015) *Biophys. Physicobiol.* 12, 121-129 [Review]., (2017) *J. Am. Chem. Soc.* 139, 4376-4389., (2017) *Sci. Rep.* 7, 44427., (2017) *Sci. Rep.* 7, 41879., (2018) *Phys. Chem. Chem. Phys.* 20, 3172-3183., (2018) *J. Phys. Chem. Lett.* 9, 2857-2862.

> IL242. Invited Lecture
Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

A NEW GROUP OF ANTARCTIC MICROBIAL RHODOPSINS WITH INWARD PROTON-PUMPING CAPABILITY

Authors: Andrew Harris[1], Ethan Watt[1], Anh Hoang[1], Michael Lazaratos[2], Ana-Nicoleta Bondar[2], Leonid Brown[1]
Presenting Author: Leonid Brown
1) University of Guelph, Ontario, Canada 2) Freie University, Berlin, Germany

We have identified a new group of microbial rhodopsins in publicly available metagenomic data (DOE JGI IMG)[1] from a lake in Antarctica. The new group has a unique amino acid sequence in which the proton acceptor position on the extracellular side of retinal is occupied by either F, L, or M, while the proton donor position on the cytoplasmic side shows mainly E (and occasionally Q). Thus, the functional helix C triad motif for this group is F/L/M-S-E/Q, not observed previously. Another interesting feature is conservation of a helix C cysteine homologous to that of the DC gate of channelrhodopsins[2]. It appears that the Antarctic rhodopsins are highly homologous to recently identified archaeal schizorhodopsins[3].

To characterize the new group, we have expressed one of its members (which we called AntR) in *E. coli* and performed ion transport assays and spectroscopic studies (visible, FTIR, Raman) in parallel with molecular dynamics simulations. Interestingly, the photochemistry and functionality of AntR are highly similar to that of xenorhodopsins, such as PoXeR[4], despite the lack of significant sequence homology. Similar to xenorhodopsins, AntR displays photochromicity and bistability, with metastable states exhibiting all-*trans*- and 13-*cis*-15-*syn* retinal, while ion transport assays on whole *E. coli* cells have shown that AntR actively transports protons in the cytoplasmic direction in response to light. The slow photocycle turnover and robust transport are consistent with a double photon transport mechanism. Vibrational spectroscopy and time resolved analysis in the visible range in combination with several mutations and MD simulations will inform the development of a detailed mechanism of proton transport for these peculiar Antarctic rhodopsins. In our opinion, it is likely that the inward proton transport plays a photosensory role.

References

1. Chen, I.A., Markowitz, V.M., Chu, K., Palaniappan, K., Szeto, E., Pillay, M., Ratner, A., Huang, J., Andersen, E., Huntemann, M., Varghese, N., Hadjithomas, M., Tennessen, K., Nielsen, T., Ivanova, N.N. & Kyrpides, N.C. IMG/M: integrated genome and metagenome comparative data analysis system. *Nucleic Acids Res* **45**, D507-D516 (2017).
2. Nack, M., Radu, I., Gossing, M., Bamann, C., Bamberg, E., von Mollard, G.F. & Heberle, J. The DC gate in Channelrhodopsin-2: crucial hydrogen bonding interaction between C128 and D156. *Photochem Photobiol Sci* **9**, 194-8 (2010).
3. Bulzu, P.-A., Andrei, A.-S., Salcher, M.M., Mehrshad, M., Inoue, K., Kandori, H., Beja, O., Ghai, R. & Banciu, H. The sunlit microoxic niche of the archaeal eukaryotic ancestor comes to light. *bioRxiv*, 385732 (2018).
4. Inoue, K., Ito, S., Kato, Y., Nomura, Y., Shibata, M., Uchihashi, T., Tsunoda, S.P. & Kandori, H. A natural light-driven inward proton pump. *Nat Commun* **7**, 13415 (2016).

> IL247. Invited Lecture

Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

THE SEARCH FOR NEW MICROBIAL RHODOPSINS USING METAGENOMICS

Authors: Oded Beja[1], Johannes Oppermann[2], Jonas Wietek[2], Peter Heggeman[2], Keiichi Inoue[3, 4], Hideki Kandori[3, 4], Alina Pushkarev[1], Andrey Rozenberg[1]
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Many organisms capture or sense sunlight using rhodopsin pigments. These rhodopsins are currently divided to two distinct protein families: type-1 (microbial rhodopsins) and type-2 (animal rhodopsins). Type-1 and type-2 rhodopsins show little or no sequence similarity to each other, as a consequence of extensive divergence from a common ancestor or convergent evolution of similar structures. Using marine metagenomes, new anion channelrhodopsins (type-1) were detected that form distinct families compared to the 3 already known cation and anion channelrhodopsin families. One of the families shows an unprecedented desensitization of the initial peak current to almost zero activity in continuous light (*1*). In addition, and this time using functional metagenomics, a previously unknown and diverse family, the heliorhodopsins, which are distantly related to type-1 rhodopsins was discovered (*2*). The orientation of heliorhodopsins in the membrane is opposite to that of type-1 or type-2 rhodopsins, with the N-terminus facing the cell cytoplasm. In addition, heliorhodopsins show photocycles longer than 1 second, suggestive of light sensory activity. In my lecture I will discuss the potential of metagenomics for future discoveries of new rhodopsin activities.

References

1. J. Oppermann*et al.*, MerMAIDs: A novel family of metagenomically discovered, marine, anion-conducting and intensely desensitizing channelrhodopsins. *Nature communications* **accepted** (2019).
2. A. Pushkarev*et al.*, A distinct abundant group of microbial rhodopsins discovered using functional metagenomics. *Nature* **558**, 595-599 (2018).

> IL244. Invited Lecture
Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

RETINAL PROTEINS IN MICROBES: DIVERSITY AND MECHANISMS

Authors: Hideki Kandori[1]
Presenting Author: Hideki Kandori
1) Nagoya Institute of Technology

When I was a graduate student, microbes containing retinal proteins (microbial rhodopsins) were only found in extreme environments such as extremely salty lakes. At that time, the functions of microbial rhodopsins were light-driven H^+ pump, Cl^- pump, and light sensors.

This view has been largely changed now. In fact, if you scoop up sea water with your hands, more than 70 % of living things have retinal proteins. Functions of microbial rhodopsins are highly diverse now; light-driven H^+ pump, Na^+ pump, Cl^- pump, SO_4^{2-} pump, light-gated cation channel, light-gated anion channel, light sensor, photoactivated enzyme, etc.

Despite wide variety of functions, structure and photochemistry are similar among microbial rhodopsins. This suggests small differences in structure and structural changes leading to each function, and mechanisms of such functional expressions are of interest. In this symposium, diversity and mechanisms of microbial rhodopsins will be discussed.

> P116. Poster

Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

A WIDE DIVERSITY OF FLUORESCENCE COLOR AND BRIGHTNESS IN MICROBIAL RHODOPSINS

Authors: Keiichi Kojima[1], Rika Kurihara[1], Masayuki Sakamoto[2], Xiaomin Zhang[2], Haruhiko Bito[2], Yuki Sudo[1]
Presenting Author: Keiichi Kojima
1) Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan 2) Department of Neurochemistry, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Microbial rhodopsin is a seven-transmembrane photoreceptive protein containing retinal as a chromophore. They are widely distributed in all domains of life (i.e., archaea, bacteria and eukarya) with a wide variety of photo-induced biological functions, such as ion pumps, ion channels and light-sensors [1]. In addition to the biological importance, microbial rhodopsin has become a focus of interest because of its applicability to the visualization tools of membrane potentials in living cells by taking advantage of their fluorescence characteristics. So far, archaerhodopsin-3 (AR3) has been mainly applied to the voltage-sensors. Here, to explore novel rhodopsin molecules showing different color and/or brightness from AR3, we quantitatively analyzed fluorescence properties of fifteen microbial rhodopsins in the same condition [2]. The fluorescence analysis indicated the wide range of excitation and emission fluorescence wavelengths from 460 to 576 nm and 620 to 715 nm, respectively. Of note, twelve rhodopsins showed 2 ~ 8-fold stronger fluorescence than AR3. The fluorescence brightness was relatively correlated with the number of Ser residues, suggesting their importance for the fluorescence brightness. Then, we analyzed the expression and fluorescence intensities of microbial rhodopsins in mouse hippocampal neurons. Among them, several rhodopsins were localized well to plasma membrane and showed comparable fluorescence intensities with AR3, suggesting their potential as new “high-fluorescent” rhodopsin-based tools for voltage-sensors in animal cells. Thus, our findings provide the molecular basis of rhodopsin-based variants with various colors and brightness, which applicable for voltage imaging.

References

[1] Kurihara & Sudo, (2015) *Biophys. Physicobiol.*, 12, 121-129.
[2] Kurihara et al., in preparation.

> P117. Poster

Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

MerMAIDs: NOVEL METAGENOMICALLY DISCOVERED, MARINE, ANION-CONDUCTING AND INTENSELY DESENSITIZING CHANNELRHODOPSINS.

Authors: Johannes Oppermann[1], Bernhard Liepe[1], Paul Fischer[1], José Flores-Uribe[4,6], Arita Silapetere[1], Enrico Peter[1], Itai Sharon[2,3], Anke Keidel[5], Johannes Vierock[1], Joel Kaufmann[7], Matthias Broser[1], Meike Luck[1], Franz Bartl[7], Peter Hildebrandt[5], Oded Béjà[4], Peter Hegemann[1], Jonas Wietek[1]
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Channelrhodopsins (ChRs) are light-gated ion channels [1], mediating photomotility in algae [2] and widely used in neurosciences to control membrane potential with light [3]. In continues bright light initial peak photocurrents of most ChRs desensitize to a steady state level with up to 70 % reduced amplitude [1], caused by the population of a parallel photocycle with an open state of low conductance [4].

Here, we present a novel, metagenomically identified family of anion-conducting ChRs (MerMAIDs: **Me**tagenomically discove**r**ed, **M**arine, **A**nion-conducting and **I**ntensely **D**esensitizing ChR**s**) that is phylogenetically distinct from all ChRs known to date. During continuous light exposure, their photocurrents almost completely desensitize, caused by the accumulation of a late non-conducting photointermediate, where the ion permeation pathway is molecularly interrupted. The desensitization of MerMAIDs can be adequately explained with a single photocycle with a short-lived conducting state followed by a desensitized state of long lifetime. Additionally, we identified a cysteine, conserved in ChRs, as a critical factor for the strong desensitization in MerMAIDs.

Our in-depth molecular and biophysical investigation of the MerMAIDs in conjunction with a simplified reaction mechanism will provide a better understanding of how ChRs work in general.

> P118. Poster

Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

FUNCTIONAL AND SPECTROSCOPIC ANALYSES OF NOVEL CYANOBACTERIAL RHODOPSINS

Authors: Masumi Hasegawa[1, 2], Keiichi Kojima[3], Yosuke Nishimura[1], Yu Nakajima[1], Yuki Sudo[3], Susumu Yoshizawa[1, 2]
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Introduction

Microbial rhodopsin (hereafter rhodopsin) is a photoreceptor protein containing retinal as a light absorbing chromophore. To date, various functions of rhodopsin, such as light-driven ion pump, light sensor, and light-gated ion channel, have been reported. Among the ion pump, an outward proton pump rhodopsin generates proton motive force and it presumably leads to activation of adenosine triphosphatase (ATP) synthase. Such rhodopsin genes are widely distributed in various microorganisms, indicating that light-driven outward proton pump is useful for their growth and survival (Gómez-Consarnau et al. 2007; DeLong and Béjà 2010). Interestingly, outward proton pump rhodopsins were also found in phototrophic cyanobacteria (Mongodin et al. 2005). This suggests that cyanobacteria would utilize light using not only oxygenic photosynthesis but also rhodopsin-mediated photosystem. Therefore, it is important to elucidate the light-utilization mechanism of cyanobacteria possessing a rhodopsin gene. In this study, we report a novel cyanobacteria-specific rhodopsin clade and its function

Materials and Methods

Sequence homology search for rhodopsins was conducted in 155 cyanobacterial genomes. The phylogenetic tree of rhodopsins was computed by RAxML version 8.2.11 (Stamatakis 2014) using 100 times rapid bootstrapping. Measurements of the ion transport activities using light-induced pH changes of *E. coli* cell suspensions expressing novel rhodopsins were performed. To investigate the photochemical properties of one of the cyanobacterial rhodopsins, N2098R, we carried out spectroscopic analysis.

Results

Phylogenetic analysis showed a new cyanobacteria-specific rhodopsin clade (cyanorhodopsin: CyR). Light-induced pH dropping of the cell suspensions was confirmed in all four CyRs, and it was abolished under the presence of a protonophore. This indicated that protons were transported from the cytoplasmic side to the extracellular side by light irradiance. To investigate the photochemical properties, the recombinant N2098R protein was purified. The absorption maximum of N2098R was located at 550 nm, and photocycle of N2098R was relatively fast (~300 ms). During proton transport, N2098R firstly releases proton and secondary uptakes proton.

Discussion

Based on these results, we concluded that N2098R functions as an outward proton pump. Judging from the absorption maximum and the time region, we identified K and M intermediates. In addition, the proton transport manner of N2098R is similar to bacteriorhodopsin (BR) (Grzesiek and Dencher 1986) but differs from proteorhodopsin (PR) (Dioumaev et al. 2002). This is the first report of the cyanobacteria-specific light-driven outward proton pump rhodopsins, which differ phylogenetically from other known light-driven outward proton pumps. The discovering of these rhodopsins in cyanobacteria provides a new insight into the way of energy utilization in phototrophic microorganisms.

> P119. Poster

Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

CHARACTERIZATION OF NOVEL RHODOPSIN FROM FRESH WATER ACTINOBACTERIA

Authors: Matthew Blain-Hartung[1,2], Lena Schaefer[1], Thomas Friedrich[1], Peter Hildebrandt[1], Katrina Forest[2]
Presenting Author: Matthew Blain-Hartung
1) Technische Universitat Berlin 2) University of Wisconsin, Madison

Actinobacterial strains of the acI lineage are often the most abundant organisms in fresh water communities and are thus presumed to be key members of the limnal environment. Recent seminal work has established that many fresh water bacteria encode and assemble rhodopsins; in fact, in Lake Mendota, the gene that encodes actinorhodopsin (*actR*) is among the most highly transcribed genes. Recent work in the Forest group has established that acI also encode the biosynthetic pathway to synthesize retinal, and that actinorhodopsin (ActR) produced in *E. coli* is a green light-activated proton-pumping rhodopsin (1). However, much is still unknown about the photochemical and biophysical properties of ActR, as well as about the biological roles this abundant protein plays in actinobacteria and in the ecophysiology of fresh water communities.

We aim to characterize the structure and function of ActR using a variety of techniques including FTIR and Raman spectroscopy, electrophysiology, and crystallographic studies. Of particular interest, the sequence of ActR predicts that this rhodopsin may bind a secondary carotenoid on the surface of the protein. As secondary carotenoids in bacterial rhodopsins with similar amino acid sequence motifs have been shown to expand the active wavelengths via fluorescence energy transfer to retinal, our group will investigate if ActR binds a secondary carotenoid and how that carotenoid binding affects the photochemical properties of the protein and its proton pumping capabilities. In particular, we aim to elucidate the detailed photocycle of ActR and document how any secondary carotenoid binding may affect its photochemical properties.

Furthermore, we are investigating the photochemical and biophysical properties of ActR in near physiological lake conditions. As fresh water lakes are incredibly dynamic systems and parameters such as pH, solutes, oxygen levels, temperature, etc. can diverge dramatically, it may be pertinent to understand the function and properties of ActR in a variety of these conditions.

Acknowledgements
Einstein Foundation

References

1. JR Dwulit-Smith, et al. - Appl. Environ. Microbiol. Nov 2018, 84 (24) e01678-18;

> P121. Poster

Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

STRUCTURAL INVESTIGATION OF THE KR2 RHODOPSIN IN ITS SODIUM-PUMPING STATE

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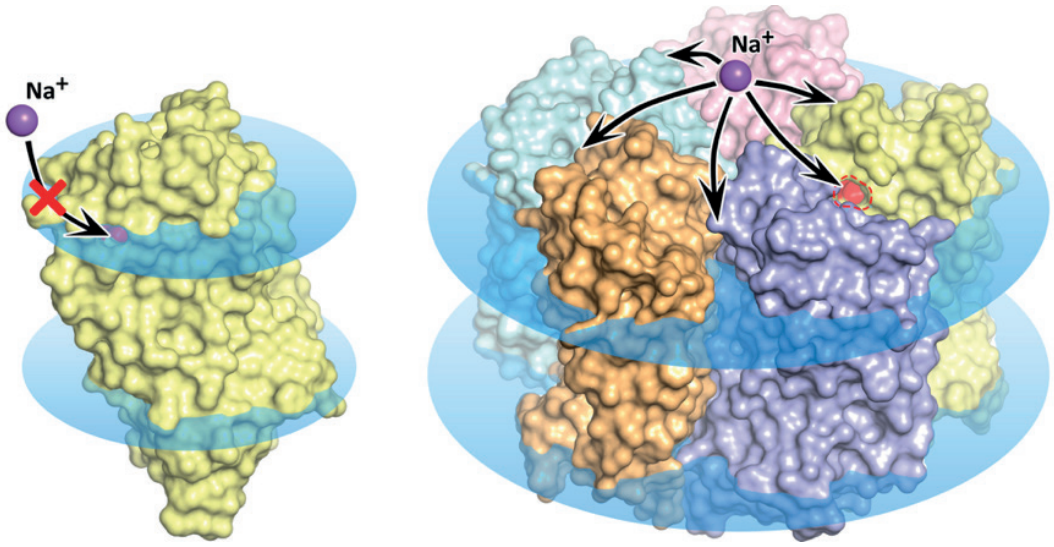
Rhodopsins are the most universal biological light-energy transducers and abundant phototrophic mechanisms evolved on Earth. They are found in all the kingdoms of life and have a remarkable diversity and potential for biotechnological applications. Recently, the first sodium-pumping rhodopsin KR2 from *Krokinobacter eikastus*, which pumps Na^+ under physiological conditions, but H^+ at acidic pH, was discovered and functionally and structurally characterized[1–3]. However, the existing structures of KR2 and suggested mechanisms of protein work are contradictory. Moreover, the crystals used for the investigations were initially grown not under physiological, but at low pH. Thus, the mechanism of Na^+ pumping is not yet completely understood. We solved the high resolution structure of KR2 at 2.2 Å using the crystals grown at physiological pH, representing its Na^+-pumping state[4]. We successfully crystallized and solved 13 supporting high resolution structures of the KR2 and its mutants, including K^+-pumping variants, in functionally important states. The structures shed light on the sodium pumping mechanism of KR2 and helped us to show that oligomerization of the microbial rhodopsin is essential for its biological function. The studies also demonstrate the rearrangements in the protein related to pH decrease and also the dehydration of the crystals. The precise structure provides new insights into the mechanisms of microbial rhodopsins and opens the way to a rational design of novel optimized cation pumps for optogenetics[5]

References

1. Inoue, K. *et al.* A light-driven sodium ion pump in marine bacteria. *Nat. Commun.* **4,** (2013).
2. Kato, H. E. *et al.* Structural basis for Na + transport mechanism by a light-driven Na + pump. *Nature* **521,** 48–53 (2015).
3. Gushchin, I. *et al.* Crystal structure of a light-driven sodium pump. *Nat. Struct. Mol. Biol.* **22,** (2015).
4. Kovalev, K. *et al.* Structure and mechanisms of sodium-pumping KR2 rhodopsin. *Sci. Adv.* **5,** eaav2671 (2019).
5. Grimm, C., Silapetere, A., Vogt, A., Bernal Sierra, Y. A. & Hegemann, P. Electrical properties, substrate specificity and optogenetic potential of the engineered light-driven sodium pump eKR2. *Sci. Rep.* **8,** 9316 (2018).



> P122. Poster

Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

REPLACEMENTS OF "H+ DONOR" RESIDUES IN THE LIGHT-DRIVEN H+-PUMP RHODOPSINS

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Many H^+-pump rhodopsins conserve "H^+ donor" residues to facilitate the H^+-transfer reactions in the cytoplasmic (CP) channels. For conventional H^+ pumps, this residue is conserved as Asp or Glu, but is replaced by Lys in the minority like *Exiguobacterium sibiricum* rhodopsin (ESR). In the dark states, both Asp and Glu donors are protonated, whereas Lys donor is deprotonated. Thus, the H^+-transfer reactions are totally different between Asp/Glu and Lys-type H^+ pumps. In the Asp/Glu type, the carboxyl donor firstly donates H^+ to the Schiff base locating the center of the protein and then captures another H^+ from the CP medium. In contrast, the Lys donor firstly captures H^+ from the CP medium and then donates it to the Schiff base. Thus, Asp/Glu and Lys-type H^+ pumps seem to have different machineries, which are probably optimized to respective H^+-transfer reactions. In this study, we examined these differences by analyzing the replacement effects of donor residues.

The Asp and Glu-type pumps are believed to have common machinery. But, they showed different responses to the donor replacements with Lys residue. Here, we observed the M-intermediate decay by the flash-induced absorbance changes. By the replacements, M-decay rate became pH dependent for Asp-type deltarhodopsin (DR) but was still pH independent for Glu-type proteorhodopsin (PR), indicating that the embedded Lys donor is not functional in DR, but functional in PR, respectively. On the other hand, the embedded Asp and Glu donors in ESR functioned well. Thus, PR seems to share common machinery with ESR but not with DR. The Asp-type bacteriorhodopsin (BR) is known to cause CP channel opening, which hydrates this channel and then drives the deprotonation of the Asp donor. Thus, we examined the hydrations by detecting the activation volumes ($\Delta V^{\ddagger}$) of M decays. DR showed large $\Delta V^{\ddagger}$ about 50 mL/mol, which is comparable with the value for BR (Váró *et al*., *Biochemistry* 1995) and reflects the hydration of the channel. On the other hand, significantly smaller $\Delta V^{\ddagger}$ (10 − 30 mL/mol) was observed for PR, ESR and their donor replacement mutants. For Glu and Lys-type pumps, thus, their CP channels appear not to be well hydrated. They might exert the H^+-transfer reactions by only the swing-like motions of the donor side chains. Due to this common machinery, their donor residues might be mutually replaceable.

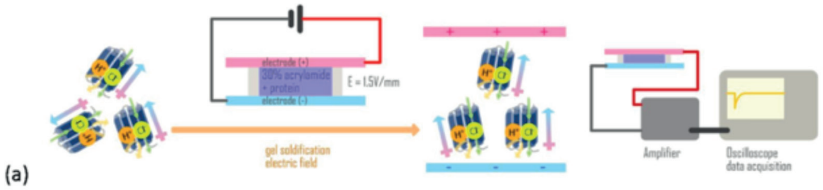
> P123. Poster

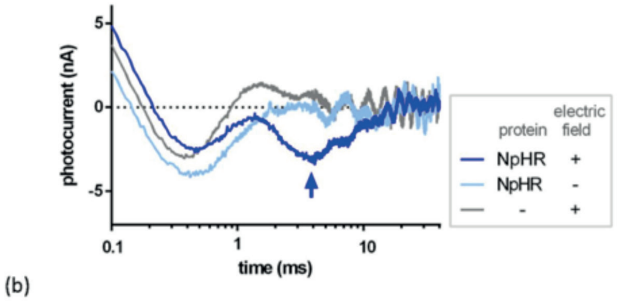
Symposium SENS-1 Retinal Proteins in Microbes: Diversity and Mechanisms (Hideki Kandori)

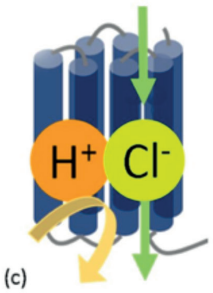
TITLE: INVESTIGATE THE MOLECULAR MECHANISM OF LIGHT DRIVEN CHLORIDE PUMPING-RELATED UNIQUE PROTON SIGNAL IN HALORHODOPSIN FROM NATRONOMONAS PHARAONIS

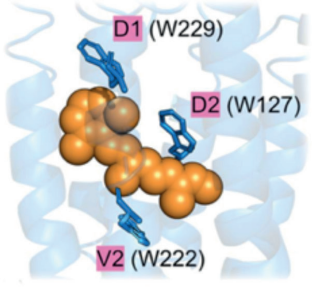
Authors: Cheng-Hong Tu[1], Chii-Shen Yang[1]
Presenting Author: Cheng-Hong Tu
1) Department of Biochemical Science and Technology, National Taiwan University

Natronomonas pharaonis belongs to the order of Halobacteriales, and was first isolated from soda lakes where it has to cope with two extreme conditions, high salt concentrations and an alkaline pH of 11. It has two kinds of microbial-rhodopsins, including halorhodopsin and sensory rhodopsin II, which functions as light-driven inward chloride pump and light-sensing photophobic response separately. Halorhodopsin is believed to maintain osmolarity and generate PMF in Archaea, while it's also widely used in the field of optogenetics to silence neurons upon light activation and to restore damaged visions. Therefore, with a prosperous future applications, it becomes more important to unveil the mechanism of its recently discovered unique proton translocation behavior. Upon previous mutagenesis study, it has been proved that proton signal is closely related to chloride transport, and the model of intracellular-side proton circulation was proposed. Among the photocycle of NpHR: HR→K→L→N→O→HR, the N state forms an intracellular water channel by its transmembrane helix F and C to facilitate chloride release. Since the water channel was composed of non-charged residues, it is postulated that a proton should facilitate the release of chloride ion in the form of HCl. However it lacks direct experimental proof up until now. In this study, we first demonstrate detailed analysis about the unique proton signal of WT-NpHR under different environment, verifying the intracellular proton circulation model. On the other hand, we are also interested in how tryptophans in the retinal binding pocket help retinal re-isomerize. By mutagenesis study, we distinguish certain tryptophans that help retinal absorb ultraviolet light around 280nm, and thus accelerate photocycle.









(e)

	Trp→Phe	Trp→Ala
D1	Functional Can not absorb 280nm to accelerate photocycle	Loss of function Blue-shifted Max Abs
D2	Loss of function Blue-shifted Max Abs Can not absorb 280nm to accelerate photocycle	Loss of function Blue-shifted Max Abs
V2	Functional	Functional Less stable

> IL248. Invited Lecture
Symposium SENS-2 Rhodopsins from Eukaryotes and their Optogenetic Application (Peter Hegemann)

MOLECULAR ENGINEERING OF MICROBIAL RHODOPSINS

Authors: Peter Hegemann[1], Johannes Vierock[1], Meike Luck[1]
Presenting Author: Peter Hegemann
1) Humboldt Universität zu Berlin, Germany

Molecular research on microbial rhodopsins has been revitalized during the recent years due to their wide optogenetic application especially in the neurosciences.

Our group studies light-driven channels, ion pumps and enzymes and we modified colour, kinetics, ion selectivity and substrate specificity. Recently we completed a comprehensive study on Chrimson, the most red shifted Channelrhodopsin. Based on a novel high-resolution crystal structure of Chrimson (2.6 Å), we investigated molecular determinants of red light activation and photocurrent kinetics of Chrimson (Oda et al. 2018). We identified key amino acids that are crucial for the counterion configuration of the retinal Schiff'base, the planarity of the retinal chromophore and the polarity of the retinal binding pocket that together determine the long wavelength absorption. Based on these mechanistic insights we engineered an even further red-shifted Chrimson mutant with fast photocurrent kinetics, peak activity at 610nm and significantly reduced blue light activation.

In another project we characterized the light-driven proton pump (CsR) of the polar alga *Coccomyxa subellipsoidea.* The crystal structure with 2.0-Å resolution enabled us to identify distinct features that determine ion transport directivity and voltage sensitivity. A specific hydrogen bond between the highly conserved Arg83 and the nearby non-conserved tyrosine (Tyr14) guided our structure-based transformation of CsR into an operational light-gated proton channel (CsR,y14E; CySeR). Our findings reveal that molecular constraints that distinguish active pumps from passive channels are structurally more confined than it was generally expected (Fudim et al. 2019). I will discuss structure-based design of novel highly ion selective optogenetic tools, which derive from microbial pumps and may be used for specific cell de- or hyper-polarization.

Alternative reaction pathways will be discussed for Channelrhodopsin-2 and for the Histidine kinase rhodopsin (HKR) of *Ostreococcus tauri* on the basis of a recently extended bi-circular reaction models (Kuhne et al. 2019)(Luck et al. 2018).

References

Oda et al. (2018) *Nature Comm.* 9(1):3949. doi: 10.1038/s41467-018-06421-9.
Fudim et al. (2019) *Sci. Signal.* 12 (537), eaav4203; doi: 10.1126/scisignal.aav4203
Kuhne, (2019) *PNAS* 116:9380-9389. doi: 10.1073/pnas.1818707116.
Luck et al. (2019) *Biochemistry* 58(14):1878-1891. doi: 10.1021/acs.biochem.8b01200

> IL249. Invited Lecture
Symposium SENS-2 Rhodopsins from Eukaryotes and their Optogenetic Application (Peter Hegemann)

RHODOPSIN CYCLASES, NOVEL CATALYSTS FOR THE OPTOGENETIC CONTROL OF CGMP AND CAMP

Authors: Matthias Broser[1], Ulrike Scheib[1], Oana M. Constantin[2], Shang Yang[3], Shiqiang Gao[3], Shatanik Mukherjee[1], Katja Stehfest[1], Georg Nagel[3], Christine E. Gee[2], Peter Hegemann[1]
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1) Institute for Biology, Experimental Biophysics, Humboldt-Universität zu Berlin, 10115 Berlin, Germany. 2) Institute for Synaptic Physiology, Center for Molecular Neurobiology Hamburg, University Medical Center Hamburg-Eppendorf, 20251 Hamburg, Germany. 3) Department of Biology, Institute for Molecular Plant Physiology and Biophysics, Biocenter, Julius-Maximilians-University of Würzburg, 97082 Würzburg, Germany.

Rhodopsin-guanylyl cyclases (RhGCs) belong to the class of enzymrhodopsins - natural rhodopsin-based photoreceptors with light-regulated enzyme activity. RhGCs were recently discovered in in the genomes of aquatic fungi from *Blastocladiomyocta* and shown to be involved in the phototaxis of fungal zoospores [1]. These proteins comprise 8 transmembrane helices with cytoplasmic N- and C-termini and a short coiled-coil linker that connects a class III guanylyl cyclase domain with the C-terminus of the rhodopsin [2]. We characterized the RhGCs from two related fungi, *Blastocladiella emersonii* and *Catenaria anguillulae*. Both proteins highly selectively produce cGMP when illuminated with green light, while no enzyme activity is observed in darkness. Transient spectroscopy on the isolated photosensors identified the main components of the rhodopsin photocycle. When heterologously expressed in various cell types, both RhGCs can be used as optogenetic tools that enable the control of cGMP-level by light [3]. The insertion of two point mutants (E497K/C566D) within the nucleotide binding site of the cyclase domain swaps the substrate specificity towards ATP and allows the generation of rhodopsin-adenylyl cyclases. Albeit these constructs exhibit some dark activity they can be used in hippocampal neurons to modulate cAMP-dependent signaling pathways. Finally, we solved the ligand-bound crystal structure of the isolated mutated cyclase domain, CaAC, at 2.25 Å resolution, which gives insight into the nucleotide binding pocket and allows assumptions about the intramolecular activation pathway of the novel photoreceptor.

References
[1] Avelar et al. (2014), Current Biology *24*, 1-7
[2] Gao et al. (2015), Nature Communications 6:8046
[3] Scheib et al. (2018), Nature Communications 9:2046

> IL250. Invited Lecture
Symposium SENS-2 Rhodopsins from Eukaryotes and their Optogenetic Application (Peter Hegemann)

EXCITED-STATE DYNAMICS IN UV AND NEAR-IR ABSORBING MICROBIAL RHODOPSINS

Authors: John Kennis[1], Yusaku Hontani[1], Srividya Ganapathy[2], Miroslav Kloz[3], Joern Weissenborn[1], Sean Frehan[1], Matthias Broser[4], Meike Luck[4], Peter Hegemann[4], Willem de Grip[2]
Presenting Author: John Kennis
1) Vrije Universiteit Amsterdam 2) Leiden University 3) ELI Beamlines, Institute of Physics, Prague 4) Humboldt University Berlin

Most microbial rhodopsins employ a protonated Schiff base retinal to trigger their light-dependent function. Here, we report on the excited-state dynamics of two microbial rhodopsins that do not fall in this category: Histidine kinase rhodopsin 1 (HKR1) and proteorhodopsin equipped with a retinal analogue.

HKR1 is a bimodal switchable microbial rhodopsin with an unprotonated retinal Schiff-base as chromophore of the UV-absorbing state. It serves as a model system of UV-absorbing animal rhodopsins, including the human OPN5. We report the photoisomerization and protonation dynamics of the HKR1 UV-state probed by transient absorption (TA) and femtosecond stimulated Raman spectroscopy (FSRS) from the femto- to submillisecond timescales. We demonstrate that energy level ordering is inverted with respect to canonical rhodopsins: photoexcitation of HKR1-UV occurs from the S_0 to the S_2 state because transition to the lower S_1 state is optically forbidden. Internal conversion to the S1 state takes place in 40 fs, after which the S_1 state evolves to ground-state photoproducts on 5 ps and 60 ps timescales. Isomerization reactions from the S_2 and S_1 states are discussed.

Near-infrared (NIR)-driven rhodopsins are of great interest in optogenetics and other optobiotechnological developments such as artificial photosynthesis and deep-tissue voltage imaging. Here we report that the proton pump proteorhodopsin (PR) containing a NIR-active retinal analogue (PR:MMAR) exhibits intense NIR fluorescence at a quantum yield of 3.3%. This is 130 times higher than native PR and 3-8 times higher than the QuasAr and PROPS voltage sensors. The NIR fluorescence strongly depends on pH in the range 6–8.5, suggesting potential application of MMAR-binding proteins as ultrasensitive NIR-driven pH and/or voltage sensors. Femtosecond transient absorption spectroscopy showed that PR:MMAR features an unusually long fluorescence lifetime of 310 ps and the absence of isomerized photoproducts, consistent with the high fluorescence quantum yield. Stimulated Raman analysis indicates that the NIR-absorbing species develop upon protonation of a conserved aspartate, which promotes charge delocalization and bond-length leveling due to an additional methyl-amino group in MMAR, in essence providing a secondary protonated Schiff base. This results in much smaller bond-length alteration along the conjugated backbone, thereby conferring significant single-bond character to the C13=C14 bond and structural deformation of the chromophore, which interferes with photoinduced isomerization and extends the lifetime for fluorescence, thus allowing a molecular understanding of the relation between absorption/emission wavelength, isomerization and fluorescence. As acidification enhances the resonance state, this explains the strong pH dependence of the NIR emission.

> IL251. Invited Lecture
Symposium SENS-2 Rhodopsins from Eukaryotes and their Optogenetic Application (Peter Hegemann)

CHANNELRHODOPSINS WITH PHOTO-CONVERTIBLE TAGS TO ACCESS A NEW DIMENSION

Authors: Matthias Prigge[1]
Presenting Author: Matthias Prigge
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Microbial rhodopsins are the pivotal photoreceptors to control neurons in an unprecedented high temporal and spatial resolution in freely behaving animals.

Time course during and after an optogenetic activation within a single neuron or an entire neuronal network is well understood, while the spatial dimension in an optogenetic experiment remains poorly defined.

Even though, theoretical approaches such as modelling light propagation in brain tissues can roughly estimate the light cone harbouring enough photons for efficient activation of neurons, the actual number of illuminated neurons out of the pool of photosensitized cells remains unknown. Additionally, different degrees of tissue compactness, ratio of white and grey matter or different absorption properties are critical parameters for light penetration, but are not well incorporated in many theoretical models. This missing volume information is introducing variability as well as making in vivo optogenetics difficult to interpret.

I therefore present here our development efforts to design a universal experimental strategy to assess the spatial dimension of an optical activation. We engineered soma-targeted channelrhodopsin together with photo-convertible fluorescence proteins. Therefore, blue light-stimulation not only triggers action potentials, but also tags neurons exposed to sufficient photon for conversion. We firstly demonstrate a largely overlap pool of photo-activated neurons and neurons labelled with the photo-converted tags. Secondly, we determine large differences in the light propagation cone in various brain areas.

Our new optogenetic probe introduces the possibility to easily readout spatial parameters in an in vivo experiment.

> IL252. Invited Lecture
Symposium SENS-2 Rhodopsins from Eukaryotes and their Optogenetic Application (Peter Hegemann)

ON-BIPOLAR CELL-TARGETED OPTOGENETIC GENE THERAPY WITH ENGINEERED MELANOPSIN-MGLUR6 CHIMERAS TO RESTORE PATTERN VISION IN MICE

Authors: Michiel van Wyk[1], Elmar Hulliger[1], Sonja Kleinlogel[1]
Presenting Author: Sonja Kleinlogel
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Introduction
Melanopsin, residing in the photosensitive ganglion cells of the retina, is a Gq- coupled class A GPCR. Melanopsin activation in ganglion cells is essential mainly for non-image forming functions such as entrainment of the circadian clock and the pupillary light reflex. Melanopsin has many favorable properties over microbial channelrhodopsins to restore vision in blind, photoreceptor-less patients: it is a human protein and possesses a 5000-fold higher light sensitivity due to the G-protein-mediated intracellular signal amplification cascade. Although retinal cone opsins and rod opsin present alternatives, their disadvantage is their dependence on constant retinal supply from the retinal pigment epithelium and thus in a photoreceptor-less retina, their rapid bleaching and response rundown.

Methods
To restore the sophisticated visual processing of the retina and potentially pattern vision in a patient, the optimal cells to target is the ON-bipolar cells, primary retinal interneurons receiving direct input from the photoreceptors via a Gi-coupled mGluR6 receptor. To turn ON-bipolar cells into “replacement photoreceptors” in a photoreceptor-less blind patient, we engineered melanopsin-mGluR6 chimeras to couple light activation to the Gi-mediated intracellular signaling cascade of bipolar cells. To deliver the optogenetic transgene efficiently and specifically to the ON-bipolar cells, we additionally designed synthetic promoters and synthetic adeno-associated viruses (AAVs).

Results
Retinal degeneration mouse lines were used to determine the ability of our designer optogenetic therapeutic to restore retinal light responsiveness and visual behavior. By a combination of electrophysiological and behavioral experiments, we could show that pattern vision at environmental daylight intensities is restored. Performing a gene therapy on post-mortem human retinal explants, efficient and specific delivery of the melanopsin-mGluR6 transgene to the ON-bipolar cells of a human retina were confirmed.

Discussion
We have engineered a therapeutic optogenetic vehicle, consisting of a human optogenetic protein; a human-sequence based ON-bipolar cell specific promoter and a synthetic AAV vector that enables efficient and specific optogene delivery to the ON-bipolar cells of the human retina restoring differential retinal signaling and behavioral pattern vision in degenerated mouse patients.

> IL253. Invited Lecture
Symposium SENS-2 Rhodopsins from Eukaryotes and their Optogenetic Application (Peter Hegemann)

METABOTROPIC OPTOGENETICS: THE NEXT GENERATION OF OPTOXRS

Authors: Elliot Gerrard[1], Alexandra-Madelaine Tichy[1,2], Harald Janovjak[1,2]
Presenting Author: Elliot Gerrard
1) Monash University, Melbourne, Australia 2) European Molecular Biology Laboratory Australia (EMBL Australia)

The field of Optogenetics aims to render cellular processes controllable with light, conferring a frame-shift improvement in temporal and spatial resolution. Historically, the majority of optogenetic tools are sourced from naturally occurring light-gated ion channels found in algae and bacteria, which allow for neuronal firing to be bi-directionally controlled with light. Recently however there is an increased interest in manipulating metabotropic signalling, i.e signalling governed by G protein coupled receptors (GPCRs), in a similar optogenetic context. To do this, a class of tools termed \'OptoXRs\' has been generated that take advantage of the modulatory and conservation of signalling domains amongst Class A GPCRs. These tools typically fuse the transmembrane and extracellular domains of mammalian rhodopsin (the opsin responsible for dim light vision in animals) with the intracellular loop(s) of a non-photic GPCR of choice (such as the beta-2-adrenergic receptor). This template has been applied to a myriad of GPCRs and has allowed for their contribution to cell and animal behaviour to be understood. We have previously utilised the modularity of this approach to determine the signalling specificity of orphan GPCRs.

The design principals of OptoXRs has not changed dramatically since their inception, with the cut sites for intracellular loop fusion fairly consistent across different receptors, guided by a series of fundamental experiments demonstrating the importance of intracellular loops 2 and 3 for G protein activation and specificity. In the meantime, the field of GPCR structure and function has advanced spectacularly, with an exponential increase in the quality and quantity of high resolution crystal structures at the core of a new degree of understanding. What comparison of these structures has told us, particularly between inactive and active states, is that the old paradigm of G protein coupling and selectivity being solely determined by intracellular loop residues is not accurate. In fact, multiple residues in the transmembrane helices play crucial roles in propagating receptor activation and recruitment of G proteins. Recent work identifying the chemical nature of conserved residue contacts between receptors has added a layer of depth to not just where these residues are but how they support receptor function. We believe these data are critical cause for a re-think of how we design chimeric receptors, with the potential to improve or even fix non-functional current designs. Outside of just modifying the fusion boundaries for OptoXRs based on recent crystal structure information, we are also entering a new era of design possibilities that utilise a wider variety of photosensitive cores in OptoXR design. The incorporation of bistable, bleach resistant, spectrally diverse or even reverse photoreceptive proteins into chimeric design have the potential to vastly improve metabotropic optogenetics. We also believe that thanks to a new appreciation of activation mechanics across different classes of GPCRs, the creation of intra-class light activated chimeras is a realistic possibility.

> IL254. Invited Lecture
Symposium SENS-2 Rhodopsins from Eukaryotes and their Optogenetic Application (Peter Hegemann)

ENGINEERING PHOTORECEPTORS INTO OPTOGENETIC TOOLS FOR THE CONTROL AND UNDERSTANDING OF CELLULAR PROCESSES IN ANIMAL AND PLANT SYSTEMS

Authors: Matias Zurbriggen[1]
Presenting Author: Matias Zurbriggen
1) Institute of Synthetic Biology and CEPLAS, Heinrich-Heine-Universität Düsseldorf, Germany

The engineering of neurons with light-regulated ion channels has enabled the non-invasive study of neuronal networks *in vivo* at unprecedented spatio-temporal resolution. This experimental breakthrough has revolutionized neurosciences, with hundreds of applications contributing key insights into nervous system function having taken root within only few years. The success of optogenetics in neurobiology is followed by the more generalized used of light as stimulus to remote control a wide range of cellular processes, from gene expression up to cell viability and function.

Our synthetic biology research focuses on engineering bacterial and plant photoreceptors sensitive to different wavelengths of the white light spectrum (UV-B, blue, green, orange, red/far-red) into synthetic photoswitches rewired to control molecular processes with high precision, quantitative and high spatio-temporal resolution, in a non-invasive way and with minimized toxicity. We implement these molecular tools into microbial, mammalian and plant cells, and *in vivo* in animals and plants for selectively manipulating signaling networks and metabolic pathways. This synthetic biology approach opens up unforeseen perspectives in fundamental and applied research, as exemplified hereby in the study of signalling pathways, biomedical field, crop design as well as for the production of high value biopharmaceuticals.

> P124. Poster

Symposium SENS-2 Rhodopsins from Eukaryotes and their Optogenetic Application (Peter Hegemann)

A CHANNELRHODOPSIN WITH A VOLTAGE-DEPENDENT AFFINITY FOR CALCIUM

Authors: Rodrigo G. Fernandez Lahore[1], Peter Hegemann[1]
Presenting Author: Rodrigo G. Fernandez Lahore
1) Humboldt-Universität zu Berlin

Channelrhodopsins (ChRs) constitute a large group of microbial opsins that exhibit light-dependent gating. Selectivity for a range of ions has been shown for natural and engineered variants, including H^+, Na^+ and Cl^--selective ChRs[1][2][3]. This repertoire of light-activated channels has allowed for the optical manipulation of neuronal activity by triggering action potentials (cation-conducting ChRs) or by inhibiting neuronal firing (anion-conducting ChRs)[4]. Considering the role of Ca^{2+} in cellular signaling processes, an optogenetic tool for the spatiotemporally defined control of calcium dynamics could be beneficial in several areas of biology. Although an improved conductance for Ca^{2+} was reported for several ChR variants, the specificity for divalent cations remains low[5][6].

Here we report a ChR with a high Ca^{2+}-affinity at negative holding potentials. Electrophysiological and calcium imaging experiments suggest similar Ca^{2+} translocation rates at physiological (~2 mM [Ca^{2+}]) and at high (70 mM [Ca^{2+}]) concentrations. When viewed in contrast to other ChRs, results indicate a vast improvement in the affinity for Ca^{2+} under negative holding potentials. This could enable photocontrol of calcium influx in cells with a high enough negative resting membrane potential (e.g. neurons).

References

[1] Vierock, Johannes, et al. “Molecular determinants of proton selectivity and gating in the red-light activated channelrhodopsin Chrimson.” *Scientific reports* 7.1 (2017): 9928.
[2] Govorunova, Elena G., et al. “Characterization of a highly efficient blue-shifted channelrhodopsin from the marine alga Platymonas subcordiformis.” *Journal of Biological Chemistry* 288.41 (2013): 29911-29922.
[3] Wietek, Jonas, et al. “Conversion of channelrhodopsin into a light-gated chloride channel.” *Science* 344.6182 (2014): 409-412.
[4] Deisseroth, Karl, and Peter Hegemann. “The form and function of channelrhodopsin.” *Science* 357.6356 (2017): eaan5544.
[5] Kleinlogel, Sonja, et al. “Ultra light-sensitive and fast neuronal activation with the Ca 2+-permeable channelrhodopsin CatCh.” *Nature neuroscience* 14.4 (2011): 513.
[6] Duan, Xiaodong, Georg Nagel, and Shiqiang Gao. “Mutated Channelrhodopsins with Increased Sodium and Calcium Permeability.” (2019).

> P125. Poster

Symposium SENS-2 Rhodopsins from Eukaryotes and their Optogenetic Application (Peter Hegemann)

EFFECT OF PH AND METAL ION ON THE ACTIVITY OF ENZYME RHODOPSIN

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Introduction

The enzyme rhodopsin is a membrane protein composed of a rhodopsin domain which binds all-*trans* retinal as a chromophore, and an enzyme domain at the C-terminal cytoplasmic side.
Here, we studied two enzyme rhodopsins, which function as guanylate cyclase (Rh-GC) and phosphodiesterase (Rh-PDE). These two molecules are expected to be novel optogenetics tools because they exhibit intracellular signaling mediators of cGMP and cAMP by light absorption. However, little is known about molecular activation mechanisms by light absorption. In addition, Rh-PDE shows constitutive activity in darkness which would be problematic when applied for optogenetics.

Methods

Enzymatic activity in mammalian cells
We expressed Rh-GC and Rh-PDE in HEK293 cells. Intercellular cGMP and cAMP were monitored by luminescence-based indicator (Glosensor assay).
Enzymatic activity in vitro
Membrane fraction was prepared after expressing Rh-GC and Rh-PDE in HEK293T cells. Activity was measured under various pH, metal conditions. Nucleotides were analyzed by HPLC.

Results and Discussion

We assessed pH dependence and metal ion dependence on enzymatic activities of Rh-GC and Rh-PDE.
Rh-GC showed a 4-fold increase in activity in the presence of Mn^{2+} compared with Mg^{2+}. This indicates that the metal ion radius significantly affect its catalytic properties.
Interestingly in Rh-PDE, the pH dependence of the hydrolysis activity of cAMP and cGMP showed asymmetry, in which cAMP hydrolysis is accelerated in lower pH whereas cGMP hydrolysis is lowered in lower pH. Histidine residues near substrate binding pocked may control the switch of activity near neutrality. Therefore, we measure mutants by focusing on the His residues and discuss the substrate selectivity of Rh-PDE based on the structural information.

Conclusions

In this study, molecular mechanism of the enzyme rhodopsin such as effect of metal ion and pH were revealed. Enzyme rhodopsins could control the second messenger. This means that they can be used in a wide range of fields such as neural firing using cyclic nucleotide-gated ion channels and elucidation of signal transduction pathways in cells. Based on this finding, we aim to develop optogenetics tools applicable to visual regeneration, neural control and apoptosis.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Yoshida, K. *et al.* (2017) A unique choanoflagellate enzyme rhodopsin exhibits light-dependent cyclic nucleotide phosphodiesterase activity. *J Biol Chem.* **292**, 7531-7541
2. Lamarche, L. B. *et al.* (2017) Purification and Characterization of RhoPDE, a Retinylidene/Phosphodiesterase Fusion Protein and Potential Optogenetics Tool from the Choanoflagellate Salpingoeca rosetta. *Biochemistry*, **56**, 5812-5822
3. Gao, S. *et al.* (2015) Optogenetic manipulation of cGMP in cells and animals by the tightly light regulated guanylyl-cyclase opsin CyclOp. *Nat Commun.* **6**, 8046

> IL255. Invited Lecture

Symposium SENS-3 Retinal Proteins in Animals, Mechanisms. Vision (Oliver Ernst)

INSIGHTS INTO THE INTERACTION BETWEEN RHODOPSIN AND ITS BINDING PARTNERS

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G-protein-coupled receptors (GPCRs) are the largest family of cell surface receptors in the human body and regulate nearly all of our physiology. The inner working of these receptors and understanding signal transfer to the G protein is therefore of immense interest. The combination of structural and biophysical approaches yields mechanistic insight into this process. The last decade has seen more than 50 GPCR crystal structures and information on GPCR dynamics is emerging from NMR, EPR and fluorescence studies. However, a bottleneck is still structure analysis of GPCRs in different functional states as well as of GPCRs upon interaction with signaling proteins. We use rhodopsin, the photoreceptor protein in vision, as a model system to understand the molecular mechanism of GPCR signaling. Site-directed spin labeling of rhodopsin and Double Electron-Electron Resonance (DEER) / EPR spectroscopy can help to fill in gaps in understanding rhodopsin conformational states. DEER spectroscopy and cryo-EM provide insight into the interaction between rhodopsin and its binding partners.

> IL256. Invited Lecture
Symposium SENS-3 Retinal Proteins in Animals, Mechanisms. Vision (Oliver Ernst)

MOLECULAR MECHANISM OF ARRESTIN BINDING TO RHODOPSIN

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Rhodopsin signaling in rod photoreceptors has long served as a prototypical GPCR-driven signaling cascade. Arrestin-1 is the key player in the two-step quenching of light-activated rhodopsin with sub-second kinetics. Arrestin-1 demonstrates 10-20-fold higher binding to active phosphorylated rhodopsin than to other functional forms. This selectivity is achieved via a “coincidence detector” type of mechanism: arrestin-1 has sensors responding to the active state of rhodopsin and rhodopsin-attached phosphates. The simultaneous engagement of both sensors induces the conformational changes in arrestin necessary for the high-affinity interaction. Mutagenesis, NMR, EPR studies, and X-ray crystallography identified both activation and phosphate sensors in arrestins and the nature of the binding-associated conformational changes. However, mutagenesis shows that the key phosphate-binding lysine in the lariat loop also plays a role in the binding of unphosphorylated rhodopsin. The interaction mechanism appears to be conserved in all arrestin family members. This information guides targeted construction of arrestins with special functional characteristics and identifies the elements that have distinct conformations in free and receptor-bound arrestins as likely docking sites for non-receptor signaling partners. DEER distance measurements between selected points in arrestin-1 and rhodopsin revealed multiple distances for each pair, indicating that the complex is dynamic and likely has different “flavors”.

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> IL257. Invited Lecture
Symposium SENS-3 Retinal Proteins in Animals, Mechanisms. Vision (Oliver Ernst)

STRUCTURAL AND FUNCTIONAL MODULATION OF ROD VISUAL RECEPTOR, RHODOPSIN BY NON-RETINOID SMALL MOLECULES DERIVED FROM NATURAL PRODUCTS

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Introduction
Rhodopsin (Rho), a visual G protein-coupled receptor (GPCR) is responsible for initiating the biochemical processes resulting in vision mediated by photoreceptors. More than hundred mutations in Rho are associated with blinding eye diseases, including currently uncurable retinitis pigmentosa (RP). Natural compounds such as flavonoids target Rho enhancing its folding and stability to correct the disease phenotype. However the underlying mechanism of their action is not fully understood. In this study, we aimed to clarify the effect of flavonoids on rod opsin stability and function.

Methods
We tested four, most common bioactive flavonoids: quercetin, myricetin and their mono-glycosylated forms. We used molecular docking to predict the binding site of these compounds within the structure of bovine rod opsin. The effect of these flavonoids on opsin stability was determined in a thermal shift assay using BFC fluorescence probe. The Trp fluorescence based G protein activation and light-induced chromophore release assays were used to assess the effect of tested flavonoids on Rho function. We also investigated the effect of flavonoids on the rod opsin oligomeric organization in cells expressing rod opsin using BRET assay, SDS-PAGE, and immunoblotting. High content imaging was utilized to determine if flavonoids improve membrane integration of RP-linked P23H Rho.

Results and Discussion
All four compounds could accommodate into the retinal-binding pocket. Additionally, quercetin and myricetin could bind to the external binding pocket. Upon binding to opsin quercetin and myricetin significantly enhanced opsin stability. Binding of these flavonoids to ligand-free opsin resulted in faster rates of chromophore entry into the binding pocket. However, these flavonoids had minor effect on Rho function. Both quercetin and myricetin increased opsin oligomerization state within the cell membrane. Binding of flavonoids to an RP-linked P23H rod opsin variant improved membrane integration of this variant *in vitro*. Together our results suggest potential of natural compound to be utilized as lead compounds in the development of novel non-retinoid therapeutics to protect retinal health in Rho-related retinal degenerative diseases.

Conclusions
Together, these studies provide evidence that flavonoids can modulate structure and properties of rod opsin, and could be beneficial in disease conditions leading to the excessive concentrations of free and misfolded opsin.

> IL258. Invited Lecture
Symposium SENS-3 Retinal Proteins in Animals, Mechanisms. Vision (Oliver Ernst)

EFFICIENCY OF ROD TRANSDUCTION ACTIVATION BY A SINGLE OPSIN MOLECULE

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Bleaching adaptation in rods is mediated by apo-opsin, which activates phototransduction with an estimated activity 10^6-fold lower than that of photoactivated rhodopsin (Meta-II). However, the actual efficiency of transduction activation by a single opsin molecule is unknown and it is unclear whether every opsin has low constitutive activity or if it exists in equilibrium between a predominant inactive state and an intermittent active state. To address this question, we sought to detect and measure the characteristics of responses produced by individual apo-opsin molecules in mouse rods with the help of electrophysiological recordings.

We studied opsin signaling in two mutant mouse strains, guanylate cyclase activating proteins knockout ($GCAPs^{-/-}$) and retinal pigment epithelium specific 65 kDa protein knockout ($RPE65^{-/-}$). First, we used $GCAPs^{-/-}$ mouse rods, which have ~5 times higher sensitivity than wildtype rods, in an effort to resolve the signal from individual opsin molecules. Prior to the recordings, dark-adapted mouse retina was dissected and small fraction of opsin was produced by bleaching <1% of rhodopsin by light. Then, the activation of the phototransduction cascade by opsin was measured from a rod outer segment by single-cell suction recordings in darkness. Surprisingly, we observed frequent photoresponse-like events in darkness from bleached $GCAPs^{-/-}$ rods. The rate of photoresponse-like events was similar from 2 hours to 12 hours after the bleach, arguing against contribution from Meta-II decay intermediates and suggesting that these events are generated by activation of the phototransduction cascade by apo-opsin. Consistent with this, dark activity returned to pre-bleached levels by regenerating bleached opsin into rhodopsin with exogenous 11-*cis* retinal treatment. To rule out any indirect activation of phototransduction by rhodopsin, we next used $RPE65^{-/-}$ chromophore-deficient rods. In this case, prior to recordings, almost all of opsin was converted into unbleachable rhodopsin by regeneration with exogenous locked 11-*cis*-7-ring retinal. The resistance of this 11-*cis*-7-ring rhodopsin to photoactivation and bleaching was confirmed biochemically. The signaling of the residual small fraction of apo-opsin in these rods was then measured with the same method above. Notably, we observed photoresponse-like events in $RPE65^{-/-}$ rods regenerated with unbleachable rhodopsin analogue, further ruling out the involvement of thermally or photo-activated rhodopsin and its decay intermediates in these photoresponse-like events. Together, our data suggests that, contrary to current beliefs, bleaching adaptation in rods is mediated by opsin that exists in equilibrium between a predominant inactive and an intermittent Meta-II like state. Notably, such Meta-II like events are generated by apo-opsin even in dark-adapted conditions and produce quantal bumps similar to these of thermally-activated rhodopsin.
Acknowledgements: This project is a collaboration between Shinya Sato and Vladimir J Kefalov (Washington University School of Medicine in St Louis), Beata Jastrzebska and Andreas Engel (Case Western Reserve University, Cleveland), and Krzysztof Palczewski (University of California Irvine).

> IL259. Invited Lecture
Symposium SENS-3 Retinal Proteins in Animals, Mechanisms. Vision (Oliver Ernst)

DARK ADAPTATION AND LIGHT PROTECTION IN VERTEBRATE VISION MOLECULAR EVOLUTION

Authors: Neda Razzagui[1], Miguel A. Fernández-Sampedro[1], Margarita Morillo[1], Pere Garriga[1]
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Rhodopsin is the photoreceptor protein of the vertebrate retina used as a landmark to study the evolution of vision at the molecular level. Here, we have conducted a functional and biochemical characterization of modern rhodopsin in three different mammal species, bovine, murine and human, in order to analyse their relationships in visual pigment evolution. These species have been selected for their relevance in vision studies, as well as by their different position on the phylogenetic tree and their diverse ethology in relation with nocturnal/diurnal life.

We have studied specific amino acid point mutations in rhodopsin, by means of combining sequence and phylogenetic analysis with the experimental study of the corresponding proteins expressed in heterologous cell cultures, in order to understand the biochemical and functional differences of visual pigments and to provide novel clues for their molecular evolution from their ancestors. To this aim, we have used UV-visible and fluoresecence spectroscopic techniques to analyze the biochemical features of the purified mutant rhodopsins.

Our spectroscopic analysis shows that the retinal release process for mouse rhodopsin (L290) is significantly slower than those of the human and bovine species (I290). This is supported by the faster retinal release rate observed for L290I mutant mouse rhodopsin, that showed a similar behavior to that of diurnal rhodopsin. This suggests a link between the activity pattern (nocturnal/diurnal) and the amino acid at this position. We propose that different Meta II decay rates could be part of a protection mechanism towards bright light exposure. The sequestration of all-*trans*-retinal by a more stable Metarhodopsin II photointermediate, arises as a possible protection mechanism in nocturnal animals, even at the cost of a worse dark adaptation. In contrast, diurnal animals would tend to better protect their visual system by mechanisms that would limit the amount of bright light reaching rhodopsin. Rod regeneration is limited, in bright light environments, by the supply of fresh 11-*cis*-retinal, but under dim-light conditions the limitation relays on all-*trans*-retinal release from photoactivated rhodopsin. In this case, a faster regeneration of the visual cycle should be expected resulting in improved dark adaptation.

An evolutionary mechanism implying a compromise between the prevalence of damage protection under bright light in nocturnal therian mammals (L290), and dark adaptation under dim light in diurnal therian mammals (I290), is proposed to have had an important influence in rhodopsin specialization.

> IL260. Invited Lecture
Symposium SENS-3 Retinal Proteins in Animals, Mechanisms. Vision (Oliver Ernst)

RHODOPSIN DYNAMICS USING AN X-RAY FREE ELECTRON LASER.

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Mammalian Rhodopsin, a prototype of “class A” G Protein-Coupled Receptors (GPCRs), the largest druggable GPCR family, is our light receptor for night vision. Upon photon absorption, it undergoes one of the fastest events in biology, which happens in the femtosecond range and triggers the isomerisation of its chromophore 11-cis retinal into all-trans. The whole rhodopsin photoactivation process lasts over about ten orders of magnitude on a logarithmic time scale, until the coupling to the G protein transducin occurs. Our recent work has focused on the determination of the structure of rhodopsin intermediates in a time-resolved manner using the laser pump and X-ray probe serial femtosecond crystallography (SFX), which has been used successfully for the prokaryotic proton pump bacteriorhodopsin [1-3]. Rhodopsin microcrystals grown in the dark are successively injected in the light of a pump laser and directly probed after various time-delays (femtoseconds to milliseconds) using an X-ray free electron laser. Several ‘static’ structures of dark [4-6] and active [7-9] states of rhodopsin have been characterized by X-ray crystallography in cryogenic conditions. However, obtaining high-resolution structures of photoactivated intermediates in a time-resolved manner and at room temperature would provide important insights on the detailed mechanism of rhodopsin activation, e.g. cis-to-trans retinal isomerization, rearrangement of amino acid side chains and water molecules, and changes in protonation states (e.g. at the E(D)RY motif).

We have now prepared and characterized crystals of wild-type mammalian rhodopsin diffracting to a resolution of 2 Å. The crystals were obtained for the first time in a lipidic cubic phase, which offers various advantages, including an optimal constant speed of sample delivery. Pilot SFX tests at the SACLA (SPring-8 Angstrom Compact Free Electron Laser) and time-resolved SFX tests at the LCLS (Linac Coherent Light Source) showed a satisfactory hit rate. Data were collected at the SACLA and SwissFEL X-ray free electron lasers. A preliminary map shows, as a proof of principle, rhodopsin with the retinal in a batho conformation at the correctly earlier-predicted time-delay. Time-resolved serial femtosecond crystallography on rhodopsin will not only give details on the molecular activation of a class A GPCR, but will also give insights into the photophysical trigger of retinal excitation upon photon absorption.

References

[1] Nogly et al. Nat Commun. (2016) 7:12314.
[2] Nango et al. Science. (2016) 354(6319):1552-1557.
[3] Nogly et al. Science. (2018) 13;361(6398).
[5] Okada et al. Journal of molecular biology. (2004) 342, 571–583.
[6] Li et al. Journal of molecular biology. (2004) 343, 1409–1438.
[7] Standfuss et al. Nature (2011) 471(7340):656-60.
[8] Choe et al. Nature (2011) 471(7340):651-5.
[9] Deupi et al. Proc Natl Acad Sci U S A. (2012) 109(1):119-24.

> IL261. Invited Lecture
Symposium SENS-3 Retinal Proteins in Animals, Mechanisms. Vision (Oliver Ernst)

iCOHERENT MULTIDIMENSIONAL STUDIES OF RHODOPSIN AND BACTERIORHODOPSIN – STRONG VIBRATIONAL NONADIABATIC COUPLING "SEES" THE LIGHT

Authors: R.J. Dwayne Miller[1, 2,]
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The relative importance of quantum effects in biological systems has long been debated. At the molecular level, the discussion reduces to the spatial and temporal coherence of the corresponding wavefunction describing the biological response function. Previous Coherent Control studies, implicating coherence transfer along the reaction coordinate, have now been complemented with 2D studies of both rhodopsin and bacteriorhodopsin that show the unusual effect of strong nonadiabatic mixing of the trans/cis states by the very modes involved in barrier crossing. We have found that the reactant/product surfaces are directly coupled by the very modes involved in the structural transition. In the case of rhodopsin, the steric repulsive forces of the excited cis conformation state impulsively direct the system to the conical intersection (CI) within a half period of the localized C11-C12 bond, the key torsional motion directing the isomerization. In bacteriorhodopsin (bR), the trans conformation allows exploration of larger nuclear configuration phase space that should lead to displacements orthogonal to the reaction pathway. In this case, we observe in the 2D spectrum very strong vibrational nonadiabatic coupling involving the key reaction modes that, by comparison to high lying theory, most strongly modulate the excited state surfaces. This mechanism acts to shape the excited state surface to dynamically refocus excited state vibration wavepackets along the reaction pathway to the CI. Despite the nearly order of magnitude difference in time scale for motion through the CI for rhodopsin and bacteriorhodopsin, the quantum yields for photoisomerization are very similar. Nature has found 2 solutions to beat rapid intramolecular vibrational redistribution processes for such high vibrational density of states that normally occur within 100 fs time scales. In both cases, there is an enormous reduction of dimensionality to a few key reaction modes. This illustrates just how highly optimized this process is as well as helps explain the highly directed nature of the associated light driven biological functions of the rhodopsin protein group. These observations indicate that quantum coherence effects can persist long enough, even along reaction coordinates, to manifest interference effects in barrier crossings. We have also analyzed model potential surfaces to highlight the importance of vibrational resonances between reactant and product surfaces leading to remarkable enhancement factors near the CI. In this context, this issue of long lived coherences will be critically examined. Based on different distinct features in 2D spectra that must be self-consistent, the evidence to date points to a role for vibrational coherences in optimization of biological functions. These results taken together posit new questions with respect to properly describing the microscopic processes involved in the photoinduced processes driving biological functions.

> P126. Poster

Symposium SENS-3 Retinal Proteins in Animals, Mechanisms. Vision (Oliver Ernst)

SPECTROSCOPIC STUDY OF PHOTOCHEMICAL REACTIONS OF A PRIMATE BLUE-SENSITIVE PIGMENT

Authors: Shunpei Hanai[1], Kota Katayama[1,2], Takuma Sasaki[1], Hiroo Imai[3], Hideki Kandori[1,2]
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1) Department of Life Science and Applied Chemistry, Nagoya Institute of Technology 2) OptoBio Technology Research Center, Nagoya Institute of Technology 3) Primate Research Institute, Kyoto University

Introduction

Color pigments are photoreceptor proteins for color vision, which contain an 11-*cis*-retinal as the chromophore. Light absorption by the retinal causes *cis-trans* isomerization, followed by conformational changes of the protein moiety. However, structural mechanisms underlying signal transduction of color pigments remain unclear mainly due to lack of structural information. We recently observed the first structural data of the primary Batho intermediate state of primate color visual pigments by low-temperature FTIR spectroscopy[1,2]. Here, we extended these studies by identifying late intermediates of color pigments, especially primate blue-sensitive pigment. Low-temperature UV-visible and FTIR spectroscopies clearly showed photochromic properties of late intermediate states at specific temperature as compared to rhodopsin for scotopic vision.

Methods

Primate blue-sensitive pigment was expressed in Sf9 insect cells, solubilized by a detergent, purified by antibody column, and reconstituted into PC liposomes. Low-temperature UV-visible and FTIR spectroscopies were applied to the hydrated films with H_2O or D_2O.

Results and Discussion

Low-temperature UV-visible spectroscopy showed that Batho intermediate converted to a BL intermediate, followed to Lumi, Meta-I, and Meta-II intermediates. Interestingly, unlike rhodopsin, each intermediate was reverted to the original state by light except for Meta-II, and such photochromic property is advantageous for structural analysis by FTIR spectroscopy. FTIR difference spectra of BL revealed that hydrogen-out-of-plane (HOOP) vibrations are diminished in comparison to Batho. As the appearance of HOOP bands is the result of retinal distortion, this result suggests that the BL of blue pigment harbors a planer all-*trans* configuration of retinal. Less distortion of the retinal in the BL intermediate will allow blue pigment to revert to original state by additional light energy.

Conclusion

We successfully identified the several intermediates converted from Batho of primate blue-sensitive pigment. We also observed the atypical photoreaction like photochromism. The first FTIR spectra of BL displayed a planer retinal structure. Further spectroscopic investigation is needed to elucidate the structural dynamics of signal transduction.

References

[1] Katayama et al., *Angrew Chem Int Ed.*, 49, 891-894 (2010).
[2] Katayama et al., *Sci. Rep.*, 45, 232-237 (2017)

> P127. Poster

Symposium SENS-3 Retinal Proteins in Animals, Mechanisms. Vision (Oliver Ernst)

STRUCTURAL STUDY OF THERMO-STABILIZED MUTATION IN CONE VISUAL OPSIN

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Introduction

Color vision is achieved by three cone opsins, blue, green, and red. Each cone opsin consists of a different opsin protein bound to a common chromophore, 11-*cis*-retinal; differential chromophore-protein interactions allow preferential absorption at a selected range of wavelengths. Structural determination of cone opsins is needed for a precise understanding of spectral tuning. The principle obstacle to solving the structures is their innate instability in detergent micelles. Here, we identify a thermostabilizing mutation of green cone opsin which confers a greater than 10-fold decrease in its rate of thermal retinal releasing compared to the wild-type opsin. FTIR spectroscopy analysis suggested that strongly hydrogen bonded water molecule is observed, which prevents the retinal Schiff base hydrolysis in the dark state. The mutationally stabilized green opsin is now applicable to crystallization.

Methods

Thermostabilized primate green-sensitive opsin was expressed in Sf9 insect cells, solubilized by a detergent, purified by antibody column, and loaded onto a SEC column. We performed vapor-phase diffusion crystallization trials.

Results and Discussion

UV-visible spectroscopy of thermostabilized green opsin exhibited 16 nm spectral blue shift in I_{max} (516 nm) as compared to wild-type (WT: 532 nm). Time-resolved UV-visible spectroscopy clearly showed a decrease in its rate of thermal retinal releasing, indicating that the hydrolysis of retinal Schiff base is prevented from the extracellular solvent. Light-induced FTIR difference spectroscopy revealed the presence of strongly hydrogen bonded water molecule that affected stabilizing of the Schiff base environment. Notably, thermostabilized green opsin showed considerable stability even in short-chain detergents such as NG or OG, which will improve the probability of success in obtaining well-diffracting crystals that are suitable for structure determination. The conventional vapor-phase diffusion method has been used successfully to crystallize different states of photoreceptive GPCR, rhodopsin. Therefore, we attempted to crystallize thermostabilized green opsin by use of this method.

Conclusion

We successfully identified the thermo-stabilized mutation of green cone opsin. Now, to determine its crystal structure, extensive crystallization trials and iterative flow of experiments will be required.

> P128. Poster

Symposium SENS-3 Retinal Proteins in Animals, Mechanisms. Vision (Oliver Ernst)

PHOTIC REGULATION OF LOCOMOTOR ACTIVITY OF COBITIDAE FISH, JAPANESE LOACH (MISGURNUS ANGUILLICAUDATUS)

Authors: Yuya Saratani[1], Keiko Okano[1], Tomonori Aoki[1], Toshiyuki Okano[1]
Presenting Author: Yuya Saratani
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Teleosts are highly diversified in marine and freshwater environment. Cypriniformes is adapted to freshwater environment, and contains two large groups (Cyprinidae and Cobitidae). Zebrafish, which belongs to the Cyprinidae family, is widely studied as a model organism. On the other hand, the Cobitidae fish species are poorly studied compared to those in Cyprinidae.

Japanese loach (*Misgurnus anguillicaudatus*) is the most common Cobitidae species in Japan and is widely distributed in East Asia. Japanese loach has been a traditional food in Japan but is nearly neglected despite its great nutritive values. In order to establish the Japanese loach as a new model fish, it would be important to understand its circadian system and photic input pathway adjusting their physiological activities.

We previously explored circadian regulation and light-responsiveness of clock gene expressions in the loach eyes. The daily expression profiles of *Cry* and *Per* mRNAs suggested that most of them are likely regulated by the internal circadian as well as environmental light signals. Japanese loach has relatively small eyes and instead uses well-developed barbels, which implies regressive function of the eyes in Japanese loach. In this study, we evaluated contribution of the eyes to the locomotor activities as the light input tissue by two physiological aproaches; characterization of retinal photopigments and measurement of their locomotor activities under various conditions.

We performed RT-PCR analysis to investigate the expression of *Opsin* genes in the eye and detected expression of some opsins, among which rhodopsin (porphyropsin) and LWS cone pigment genes are highly transcribed. Consistently, spectroscopic analysis of opsins extracted from the eyes indicated the dominant expression of porphyropsin. Amount of rod opsin is relatively lower than mice, a nocturnal animal that dominantly uses touch sense. Then, we measured their locomotor activities, which were higher in the nighttime than the daytime and regulated strongly and weakly by light and circadian clock, respectively. The regressive nature of the loach eyes further prompted us to measure the locomotor activities of the blinded fish. In spite of the circadian and photoresponsive function of the eye, removal of the eyes gave no effect on their nocturnal locomotor activity.

These results support that the loach eyes are photosensitive circadian tissue but small and likely regressive for vision, and that extraocular photoreceptor(s) would operate for the photic control of their nocturnal behavior instead of or in parallel with the eyes. Generally, photosensitivities of the ocular and extraocular photosystems are diverged and may functions cooperatively to regulate the physiological responses. In order to evaluate those systems in individual animals, we now comparing the locomotor activities under rectangular and simulated natural light-dark cycles.

> IL264. Invited Lecture
Symposium SENS-4 Non-visual and non-classical animal photoreceptors (Akihisa Terakita)

NON-VISUAL AND NON-CLASSICAL ANIMAL PHOTORECEPTORS: CONTRIBUTION OF BISTABLE NATURE OF OPSIN TO NON-VISUAL PHOTORECEPTION IN TELEOST PINEAL ORGANS

Authors: Akihisa Terakita[1]
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Animals capture environmental light and utilize the light information for not only vision but also non-visual function, e.g. light-regulation of biological rhythms. Accumulated evidence demonstrates that vertebrate non-visual photoreception employs various kinds of opsins. Multiple non-visual opsins including melanopsin (Opn4) are expressed in several kinds of retinal ganglion cells of the inner retina in mammals. In non-mammalian vertebrates, multiple non-visual opsins are localized several organs/tissues including eyes, pineal organs, brain, and skin and involved in different physiologies. Additionally, cryptochromes, which bind to a flavin as a chromophore serves as a circadian photoreceptor protein in some insects and its possible photoreception in vertebrate non-visual function has been discussed. In this symposium, structure, signal transduction and function of non-visual photoreceptive proteins are discussed.

I would like to introduce non-visual wavelength discrimination based on a pineal opsin, parapinopsin alone in teleost pineal organs. In lower vertebrates, the pineal organ, which is one of the most developed non-visual photoreceptive organs, expresses some pineal specific opsins. Parapinopsin is a UV-sensitive pineal opsin and has a bistable nature [1, 2]; that is, parapinopsin converts to the photoproduct having its absorption maximum at visible region by UV-light absorption and reverts to the original dark state having its absorption maximum at UV-region by subsequent visible light absorption, indicating that parapinopsin forms different photoequilibrium state depending on wavelength of light. Calcium imaging with zebrafish mutants revealed that the single pineal photoreceptor cells generate color opponency based on the parapinopsin interconvertibility [3]. Because other non-visual opsin-based pigments also exhibit such bistable nature, a possible contribution of bistable nature to color opponency is discussed.

References

[1] Koyanagi, Kawano et al., PNAS 101, 6687-6691 (2004)
[2] Koyanagi, Wada et al., BMC Biology13, 73 (2015)
[3] Wada et al., PNAS 115, 11310-11315 (2018)

> IL262. Invited Lecture
Symposium SENS-4 Non-visual and non-classical animal photoreceptors (Akihisa Terakita)

ERTEBRATE CRYPTCHROMES: MULTIPLE ROLES IN PHOTORECEPTION, PHOTOPERIODIC TIME MEASUREMENT, AND MAGNETORECEPTION

Authors: Toshiyuki Okano[1]
Presenting Author: Toshiyuki Okano
1) Waseda University

Cryptochromes (CRYs) are blue light photoreceptors found in a wide range of organisms and form a large protein family with photorepair enzyme photolyases (PHRs) [1,2]. The CRY/PHR family genes are structurally classified into several groups in animals. They have evolved through multiple gene duplication events, but their functional difference and redundancy are not fully understood. To answer these questions, we have been comparatively investigating spatiotemporal expression profiles and light-responsiveness of those mRNAs as well as functional analysis of those CRY proteins.

Animal-type CRYs (CRY1 and CRY2) and fruit fly CRY (dCRY) play the important roles in the circadian clock oscillator [2]: The animal-type CRY1 and CRY2 function as transcriptional repressors in the core loop of circadian clock oscillation system, while dCRY and the other non-mammalian CRYs serve as blue light photoreceptor molecules using FAD as its chromophore. In contrast to mammals that have only the two Cry genes (e.g. *mCry1* and *mCry2* in mice), nonmammal vertebrates have additional *Cry* paralogs: Fish species have multiple paralogs of the CRY1 and CRY2, among which animal-type CRY2 (called as CRY3) may play a role for the lunar timer in a tropical fish, Goldlined spinefoot (*Siganus guttatus*) that synchronizes its spawning around the first quarter moon [3-5]. Zebrafish have four paralogs of the animal-type *Cry* genes, *Cry1a/1b/2a/2b*, among which *Cry1b* shows photoperiod-dependent expression exclusively in the eyes, suggesting that *Cry1b* may be involved in a photoperiodic response in the retina. Another group *Cry4* is found in all classes of nonmmamal vertebrates, and CRY4 proteins are speculated to be a photoreceptor and/or magnetoreceptor in birds [6-8]. Recombinant CRYs are expressed in the yeast and purified to reveal the photocycle of CRYs [8].

References

[1] Lin and Todo, Genome Biol 6, 220 (2005); [2] Chaves et al., Annu Rev Plant Biol 62, 335 (2011); [3] Fukushiro et al., PLoS One 6, e28643 (2010); [4] Toda et al. PLoS One 9, e109119 (2014); [5] Takeuchi et al. Sci Rep 8, 6208 (2018); [6] Kubo et al. J Neurochem 97, 1155 (2006); [7] Watari et al. J Biol Chem 287, 42634 (2012); [8] Mitsui et al. Biochemistry 54, 1908 (2015).

> IL267. Invited Lecture

Symposium SENS-4 Non-visual and non-classical animal photoreceptors (Akihisa Terakita)

FROM THE STRUCTURE OF VERTEBRATE AND INVERTEBRATE RHODOPSINS TO NEW APPLICATIONS IN OPTOGENETICS

Authors: Gebhard F.X. Schertler[1+2]
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In this project, we explore the use of light-sensitive G protein-coupled receptors (GPCRs) –opsins– for the development of new optogenetic tools to control cellular signalling processes using light: opto-GPCRs. In a first stage we identified several new opsins capable of GPCR pathways. We extensively characterized the most promising candidate opsins biochemically in cellular assays and finally in vivo. We developed the basis for engineering bistable opsins towards more effective optogenetic tools. For this, we determined the first structure of a recombinant invertebrate rhodopsin, carried out a detailed study of the chromophore binding site with advanced biophysical methods. We were able to compare in detail monostable and bistable visual pigments. The bistable pigments in several aspects are closer to the ligand binding pharmacologically relevant family A GPCRs. In a successful engineering attempt, we were able to identify mutations that shift the wavelengths of an invertebrate rhodopsin towards the infrared. This is important for the penetration of the light into tissues. The engineered opto-GPCRs are an important alternative to the channel opsins related optogenetic tools and they have a wide range of applications that is not restricted to neurons.

References

T. Nagata, M. Koyanagi, R. Lucas and A. Terakita: An all-trans-retinal-binding opsin peropsin as a potential dark-active and light-inactivated G protein-coupled receptor. Sci. Rep. 8, 3535 (2018)

E. Gerrard, E. Mutt, T. Nagata, M. Koyanagi, T. Flock, E. Lesca, G. F. X. Schertler, A. Terakita, X. Deupi, R. Lucas: Convergent evolution of tertiary structure in rhodopsin visual proteins from vertebrates and box jellyfish. Proceedings of the National Academy of Sciences (USA) 115(24):6201-6206 (2018)

T. Nagata, M. Koyanagi, H. Tsukamoto, E. Mutt, G. F. X. Schertler, X. Deupi, A. Terakita: Rearrangement of the counterion–retinylidene Schiff base interaction upon light activation of an invertebrate rhodopsin., submitted.

D. Ehrenberg, N. Varma, X. Deupi, M. Koyanagi, A. Terakita, G.F.X. Schertler, J. Heberle, E. Lesca: The two-photon reversible reaction of the bistable jumping spider rhodopsin-1., submitted

N. Varma, E. Mutt, 1, J. Mühle, V. Panneels, A. Terakita, X. Deupi, P. Nogly, G.F.X. Schertler, E. Lesca: Crystal structure of a light-sensitive bistable class A GPCR., submitted_

> IL266. Invited Lecture
Symposium SENS-4 Non-visual and non-classical animal photoreceptors (Akihisa Terakita)

OPSINS IN THE MAMMALIAN INNER RETINA

Authors: Jessica Rodgers[1], Steve Hughes[2], Stuart Peirson[2], Mark Hankins[2]
Presenting Author: Jessica Rodgers
1) University of Manchester 2) University of Oxford

Intrinsically photosensitive retinal ganglion cells (ipRGCs), located in the mammalian inner retina, express the opsin photopigment melanopsin. Melanopsin (*Opn4*) is involved in a range of important non-image forming behaviours, including circadian photoentrainment and pupil light responses. Yet, compared to the rod and cone opsins of the outer retina, comparatively little is known about the impact of naturally-occurring *Opn4* genetic variation on melanopsin protein function and downstream physiological responses to light. We used a combination of *in vitro* live cell assays and *in vivo* expression systems to characterise the functional phenotypes of human melanopsin missense mutations. We screened 96 non-synonymous melanopsin mutants found in the NCBI Short Genetic Variation database (dbSNP) using sequence alignments and comparative approaches to select 16 potentially deleterious variants for functional characterisation using fluorescent calcium imaging in Hek293T cells. We identified a number of previously uncharacterised mutations that resulted in non-functional or attenuated melanopsin-driven responses to light. We also validated an *in vivo* mouse model of two human melanopsin polymorphisms, P10L and T394I, which have been associated with abnormal non-image forming behaviours. Intraocular injections were used to deliver floxed adeno-associated viruses containing the human melanopsin variant to the retinas of melanopsin knockout mice expressing Cre-recombinase in ipRGCs. Behavioural testing pre- and post- injection revealed that ipRGC-specific expression of either P10L or T394I was able to functionally rescue pupil light responses and circadian phenotypes, comparable with wildtype human melanopsin. Multi-electrode array recordings of virally-treated retinas revealed that ipRGCs expressing the T394I variant exhibited responses with decreased sensitivity compared to the human melanopsin positive control. Collectively, these data represent the first screen of human melanopsin genetic variants and describe several with abnormal functional properties. This could help identify individuals with altered melanopsin-driven light perception and may potentially highlight those at risk of sleep disturbance, circadian dysfunction and visual abnormalities.

> IL268. Invited Lecture

Symposium SENS-4 Non-visual and non-classical animal photoreceptors (Akihisa Terakita)

PHOTOTRANSDUCTION PATHWAYS IN INTRINSICALLY-PHOTOSENSITIVE RETINAL GANGLION CELLS (IPRGCS)

Authors: Zheng Jiang[1]
Presenting Author: Zheng Jiang
1) Baylor College of Medicine, Department of Opthalmology

Introduction

Non-image-forming visual functions, such as pupillary light reflex and circadian photoentrainment, are mediated primarily by melanopsin-expressing, intrinsically-photosensitive retinal ganglion cells (ipRGCs). They are classified into five subtypes (M1 to M5). In mouse M1-ipRGCs, by far the best-studied subtype, melanopsin activates PLCβ4 (phospholipase C-β4) to open TRPC6,7 channels, mechanistically similar to phototransduction in fly rhabdomeric (microvillous) photoreceptors. Phototransduction in the other subtypes of ipRGCs has not been explored.

Methods

The photocurrents of M2- and M4-ipRGCs were recorded by whole-cell, patch-clamp recording from flat-mount mouse retina in the presence of synaptic blockers. Photo-uncaging experiments used intracellular caged cyclic-nucleotide delivered from a whole-cell pipette. To disrupt HCN channel function, mouse retina was infected by intravitreal injection of AAV2 virus carrying a mutant HCN channel subunit. Animal circadian photoentrainment was monitored from wheel-running activity. PLR (pupillary light reflex) was recorded as described in Xue et al., Nature 479: 67-73, 2011.

Results

We found that M2- and M4-ipRGCs had a persistent intrinsic photocurrent in *Plcβ4*$^{-/-}$*or Trpc6,7*$^{-/-}$ genotype, even in *TRPC1,3,4,5,6,7*$^{-/-}$genotype. This photocurrent was insensitive to Ruthenium Red, a wide-spectrum TRPC-channel blocker, but completely blocked by ZD7288, an HCN-channel blocker. The voltage dependence of the *Trpc6,7*$^{-/-}$photocurrent was consistent with HCN-channel properties, and its amplitude was positively correlated with the hyperpolarization-induced I_h current. Immunostaining and/or genetic labeling also revealed HCN4-channel, but not CNG channel, expression in retinal melanopsin-positive cells. A virally-expressed mutant HCN channel subunit significantly reduced the light responses of M2- and M4-ipRGCs. We found that phototransduction in M1-ipRGCs, but not M2- or M4-ipRGCs, depends on Gαq, Gα11and Gα14. Photo-uncaged cyclic nucleotide in *Opn4*$^{-/-}$ M2- or M4-ipRGCs induced an inward current similar to the melanopsin-mediated response. Finally, *Trpc6,7*$^{-/-}$;rd*/rd* mice (which have lost rod/cone signals and TRPC6,7-dependent signals in ipRGCs) still exhibited PLR and circadian photoentrainment, although both functions were partially impaired.

Conclusions

We discovered that mouse M4-ipRGCs rely on a different and hitherto undescribed melanopsin-driven, ciliary phototransduction mechanism involving cyclic nucleotide as the second messenger but the activation of an HCN channel instead of a CNG channel as found in rods and cones. These findings reveal a complex heterogeneity in phototransduction among ipRGCs and, more importantly, break a general dogma about segregation of the two phototransduction motifs.

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Symposium SENS-4 Non-visual and non-classical animal photoreceptors (Akihisa Terakita)

PHYSIOLOGICAL FUNCTION OF NON-CLASSICAL PHOTORECEPTORS IN VERTEBRATE RETINA: RETINAL PHOTORECEPTORS REGULATING LIGHT-INDUCED BODY COLOR CHANGES IN ZEBRAFISH

Authors: Daisuke Kojima[1]
Presenting Author:Daisuke Kojima
1) Department of Biological Sciences, School of Science, The University of Tokyo

Many cold-blood vertebrates darken or lighten their body colors in response to ambient light intensities. Such a light-induced body color change, also called \'background adaptation\', is mediated by ocular photoreception in teleost [1, 2]. Previous studies demonstrated that classical retinal photoreceptor cells, rods and cones, are not required for background adaptation in larval zebrafish (*Danio rerio*) [3]. The light-induced behaviors thus involve non-rod non-cone retinal photoreceptor cells, possibly the ones expressing non-visual opsins such as VAL-opsins and melanopsins [4-7]. To investigate the light signaling process, we first determined the spectral sensitivity for the light-induced body color change in the wild-type zebrafish at five days old. The estimated action spectrum revealed that two kinds of spectrally distinct opsin-type molecules, tentatively termed P416 and P470, could mediate the photic regulation. In conditional and selective ablation experiments of rods and cone in the larvae, the rod/cone-ablated larvae exhibited a significantly decreased level of body color change in response to 420-nm violet light, but not to 500-nm green light. The wavelength dependency suggested that P416 is present in rods and/or cones whereas P470 is located in non-rod non-cone retinal neurons. Consistently, pharmacological treatment with a melanopsin inhibitor significantly reduced the larval response to the 500-nm green light, but not to the 420-nm violet light, in the body color change. Subsequent genetic analyses of knock-out mutant larvae confirmed that P470 is a melanopsin-type photoreceptive molecule present in a subtype of inner retinal neurons. In the retina, this P470 gene exhibited a dorsally biased expression pattern, being consistent with its role in background adaptation. In summary, the background adaptation of larval zebrafish is likely to be regulated by multiple types of photoreceptive molecules present in both *classical* and *non-classical* retinal photoreceptor cells.

References

1. Frisch (1911) *Pflüger's Arch* 138:319–387.
2. Shiraki *et al.* (2010) *Photochem Photobiol Sci* 9:1498–504.
3. Muto *et al.* (2005) *PLoS Genet* 1:e66.
4. Kojima *et al.* (2000) *J Neurosci* 20:2845–51.
5. Kojima *et al.* (2008) *J Neurochem* 104:1364–71.
6. Matos-Cruz *et al.* (2011) *PLoS One* 6:e25111.
7. Davies *et al.* (2011) *Cell Mol Life Sci* 68:4115–32.

> IL265. Invited Lecture

Symposium SENS-4 Non-visual and non-classical animal photoreceptors (Akihisa Terakita)

RETINAL MULLER GLIAL CELLS IN THE DEVELOPING RETINA OF BIRDS EXPRESS THE NON-VISUAL OPSIN OPN3 AND RESPOND TO BLUE LIGHT

Authors: Mario Eduardo Guido[1], Maximiliano N. Rios[1], Natalia Marchese[1]
Presenting Author: Mario Eduardo Guido
1) CIQUIBIC CONICET Universidad Nacional de Córdoba, Cordoba, Argentina

The retina of birds contains different types of photoreceptors involved in image and non-image forming activities: the visual photoreceptor cells (cones and rods) and the melanopsin-expressing cells (intrinsically photoresponsive retinal ganglion cells –ipRGCs- and horizontal cells). In addition, the nonvisual opsins Opn3 (encephalopsin/panaopsin) and Opn5 (neuropsin) have been found to be expressed in the vertebrate inner retina, responding to blue (BL) and UV light, respectively. Diverse retinal processes are regulated by light, among them is the functioning and adjusting of the retinal circadian clock that temporally controls retinal physiology. Here we evaluated the expression, localization and possible light regulation of Opn3 and Opn5 in the developing retina at different embryonic days (E) in the whole chick retina as well as in primary cultures of Müller glial cell (MC), by PCR, immunochemistry and fluorescence calcium imaging. Opn3 and Opn5 mRNAs and proteins appeared as early as E7-10, in the developing RGC layer and glial cells that extend throughout the forming nuclear layer. At E15, and later on –up to post-natal day 10-, a significant increase in both opsins' levels was observed in inner retinal cells, together with expression of the glial marker glutamine synthetase (GS). Opn3 and Opn5 were found to be expressed in primary neuronal and MC cultures prepared as early as at E8 and kept for 2 weeks. Significant but opposite effects of BL exposure on Opn3 expression levels and subcellular localization were observed in neuronal and MC cultures: BL substantially affected Opn3 expression in RGCs, promoting a decrease in protein levels and a change in subcellular localization away from processes, whereas in MCs, BL significantly increased its expression and modified its nuclear location. More importantly, a subpopulation of MCs responded to brief BL pulses by increasing intracellular Ca^{2+} levels. In addition, these cells also significantly changed their cellular area in response to BL. Taken together; our results show that these two opsins are expressed in inner retinal cells at early developmental stage, in both neurons and MCs of the chicken retina, with protein levels strongly regulated by light at early stages in which no-sign of vision may occur. The novel photic response observed in MCs allows us to infer that an important role is likely played by these cells in retinal physiology during the day or after light exposure.
Acknowledgements: Supported by ANPCyT-FONCyT, PICT 2013 Nº 021 and PICT 2016 Nº 187, CONICET (PIP 2014) and SeCyT-UNC.

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Symposium SENS-4 Non-visual and non-classical animal photoreceptors (Akihisa Terakita)

ANALYSIS OF OSCILLATION PATTERN IN MRNA EXPRESSIONS OF NEUROSECRETORY PROTEIN GL AND NEUROSECRETORY PROTEIN GM IN MICE

Authors: Atsuki Kadota[1], Eiko Iwakoshi-Ukena[1], Kenshiro Shikano[1], Takaya Saito[1], Yuki Narimatsu[1], Kazuyoshi Ukena[1]
Presenting Author: Atsuki Kadota
1) Hiroshima University

Introduction

We recently discovered two novel cDNAs encoding the precursor of a small secretory protein in the hypothalamus of birds and rodents. The small proteins were named neurosecretory protein GL (NPGL) and neurosecretory protein GM (NPGM), respectively. We found that NPGL and NPGM stimulated food intake and fat accumulation in rats and mice (1,2). Although there is close relationship between metabolism and biological clock, whether *Npgl* and *Npgm* have rhythm remains to be clarified. Therefore, we investigated daily oscillation of mRNA expressions of those proteins.

Methods

Male mice at 5 weeks of age were entrained to a 12h light:12h dark cycle with ad libitum normal chow food for 3 weeks before being randomly assigned to ad libitum feeding group (ALF) or restricted feeding group (RF). The RF group had access to food for 3 hour during the light phase, from zeitgeber time (ZT) 3 to ZT 6 where ZT 0 denotes light on.

After 2 weeks on the feeding paradigm, animals were sacrificed and mediobasal hypothalamus (MBH), which *Npgl* and *Npgm* are expressed specifically, was collected every 3 hr over 24h. Those mRNA expressions were measured using real-time RT-PCR.

Results

The peak of *Npgl* mRNA expression in the MBH was found at around ZT 15, and that of *Npgm* was observed at around ZT 18 under ALF. On the other hand, the peak of both mRNA expressions was detected at around ZT 3 (just before feeding) under RF.

Discussion

The results of oscillation patterns of *Npgl* and *Npgm* mRNA show that the mRNA expressions of both precursors have rhythm. The patterns may be related to the stimulation of food intake and fat accumulation by NPGL and NPGM, because the peak of expressions is presented in the dark period which mice perform feeding behavior and lipogenesis.

From the result of peak shift of mRNA expressions to around ZT 3 under RF, it is possible that the mRNA expression is altered by RF and is related to food anticipatory activity. Therefore, NPGL and NPGM may be predictable factors for timing of feeding.

Conclusions

The mRNA expressions of *Npgl* and *Npgm* have rhythmicity and are altered by timing of feeding to adapt feeding activity and fat accumulation.

Conflicts of Interest

The authors declare that no competing interests exist.

References

(1) Iwakoshi-Ukena E et al., Neurosecretory protein GL stimulates food intake, de novo lipogenesis, and onset of obesity. eLife. 2017;6:28527.
(2) Matsuura D et al., Neurosecretory protein GL, a hypothalamic small secretory protein, participates in energy homeostasis in male mice. Endocrinology. 2017;158:1120-1129.

> P130. Poster

Symposium SENS-4 Non-visual and non-classical animal photoreceptors (Akihisa Terakita)

COLOR DEPENDENT REGULATION OF CELL RESPONSES BY A NON-VISUAL BISTABLE OPSIN PARAPINOPSIN

Authors: Mitsumasa Koyanagi[1], Seiji Wada[1], Baoguo Shen[1], Takashi Nagata[1], Akihisa Terakita[1]
Presenting Author: Mitsumasa Koyanagi
1) Graduate School of Science, Osaka City University

Pineal organs of lower vertebrates such as lamprey and most teleosts discriminate UV and visible light. We previously found that a pineal opsin parapinopsin is UV-sensitive, suggesting the molecular basis of UV-reception in color opponency of the lamprey pineal organ [1]. Interestingly, parapinopsin is a bistable opsin: exposure of dark-adapted parapinopsin (inactive state) to UV light produces a stable photoproduct (active state) that is itself maximally sensitive to visible light and, on light absorption, can revert to the original dark state, showing photo-interconvertibility between the inactive and active states [1-3]. Therefore, parapinopsin has two stable “color states” with their absorption maxima at largely separated wavelengths, unlike other vertebrate cone opsins whose photoproduct is unstable. We recently discovered by calcium imaging of parapinopsin-expressing pineal cells of series of transgenic zebrafish that the spectrally distinct parapinopsin states allow this opsin alone to provide the color sensitivity of the teleost pineal organ [4]. Here we examined the performance of parapinopsin for regulating cellular responses in a color dependent manner using heterologous expression systems.

References

[1] Koyanagi et al., PNAS 101:6687–6691 (2004)
[2] Wada et al., PLoS One 7:e39003 (2012)
[3] Koyanagi et al., BMC Biol. 13:73 (2015)
[4] Wada et al., PNAS 115:11310-11315 (2018)

> P131. Poster

Symposium SENS-4 Non-visual and non-classical animal photoreceptors (Akihisa Terakita)

ANALYSIS OF PHOTOPERIODISM OF NEUROSECRETORY PROTEIN GL IN HAMSTER

Authors: Yuki Narimatsu[1], Eiko Iwakoshi-Ukena[1], Atsuki Kadota[1], Kimberly J Jennings[2], George E Bentley[2], Lance J Kriegsfeld[2], Kazuyoshi Ukena[1]
Presenting Author: Yuki Narimatsu
1) Hiroshima University, Japan 2) University of California at Berkeley, USA

Introduction

We have recently discovered a novel cDNA encoding the precursor protein termed neurosecretory protein GL (NPGL) in the hypothalamus of rodents (1). The previous study shows that NPGL increases food intake and the mass of white adipose tissue (WAT) in rats and mice (1, 2). However, it has not been clarified whether the mRNA expression of *Npgl* has photoperiodism. It is well known that hibernators such as hamsters accumulate fat as an energy source for wintering. Therefore, we investigated the expression of *Npgl* mRNA in hamsters under long-day and short-day photoperiods.

Methods

Adult female Syrian and Siberian hamsters were used in this study. They were kept under a long-day photoperiod (LD; 16 h light, 8 h dark) before the experiment. Thereafter, they were divided into two groups; one group was maintained under long-day photoperiod, and the other group was transferred to short-day photoperiod (SD; 8 h light, 16 h dark) for 12 weeks. Total RNA of the mediobasal hypothalamus (MBH) was extracted and reverse transcribed. The sequence of NPGL precursor cDNA was determined as previously described (2). The mRNA expressions under LD or SD were measured using real-time RT-PCR.

Results

We identified partial cDNAs sequence encoding NPGL by using total RNA from the MBH. In Syrian hamster, body mass and *Npgl* mRNA expression was not different between LD and SD. On the other hand, in Siberian hamster, body mass and the expression of *Npgl* mRNA were significantly reduced under SD.

Discussion

It is possible that NPGL play an important role to adapt the photoperiodism in Siberian hamster. The reduction in not only day length but also body mass may decrease the *Npgl* mRNA expression in hamster. In addition, the expression of *Npgl* may response to melatonin which is secreted from the pineal organ and changes in day length in hamster.

Conclusion

In Siberian hamster which is one of hibernators, the decrease of the body mass may be related to attenuation of the *Npgl* expression. We are analyzing the photoperiodism of NPGL using melatonin-responsive mice.

Conflicts of interest

The authors declare that no competing interests exist.

References

(1) Matsuura D et al. Neurosecretory protein GL, a hypothalamic small secretory protein, participates in energy homeostasis in male mice. Endocrinology. 2017; 158: 1120-1129
(2) Iwakoshi-Ukena E et al. Neurosecretory protein GL stimulates food intake, de novo lipogenesis, and onset of obesity. eLife. 2017; 6: 28527.

> IL275. Invited Lecture

Symposium SENS-5 Blue Light and UV receptors. In Memoriam Winslow Briggs (John Christie)

ENGINEERING THE PHOTOTROPIN PHOTOCYCLE TO MODULATE PLANT GROWTH

Authors: John Christie[1]
Presenting Author: John Christie
1) University of Glasgow

The ability to enhance photosynthetic capacity remains a recognized bottleneck to improving plant productivity. Phototropin blue light receptors (phot1 and phot2) optimize photosynthetic efficiency in *Arabidopsis thaliana*by coordinating multiple light-capturing processes. In this study, we explore the potential of using protein engineering to improve photoreceptor performance and thereby plant growth. We demonstrate that targeted mutagenesis can decrease or increase the photocycle lifetime of *Arabidopsis*phototropins *in vitro* and show that these variants can be used to reduce or extend the duration of photoreceptor activation *in planta*. Our findings show that slowing the phototropin photocycle enhanced several light-capturing responses, whilst accelerating it reduced phototropin's sensitivity for chloroplast accumulation movement. Moreover, plants engineered to have a slow-photocycling variant of phot1 or phot2 displayed increased biomass production under low light conditions as a consequence of their improved sensitivity. Together, these findings demonstrate the feasibility of engineering photoreceptors to manipulate plant growth and offer additional opportunities to enhance photosynthetic competence, particularly under suboptimal light regimes.

> IL272. Invited Lecture

Symposium SENS-5 Blue Light and UV receptors. In Memoriam Winslow Briggs (John Christie)

DETECTING LIGHT DIRECTION IN THE HYPOCOTYL OF ARABIDOPSIS THALIANA

Authors: Christian Fankhauser[1], Anupama Goyal[1], Ganesh Nawkar[1]
Presenting Author: Christian Fankhauser
1) Center for Integrative Genomics, University of Lausanne, CH-1015 Lausanne, Switzerland

The phototropin family of photoreceptors is present in all land plants to optimize photosynthesis through a number of physiological et cell biological adaptations. In Arabidopsis thaliana these include positive phototropism of stem-light structures, leaf flattening, regulation of stomatal aperture and chloroplast movements (1). Phototropic responses require the ability to detect the direction of incoming light. In Arabidopsis light sensing and the response to directional light cues both occur in the hypocotyl (the embryonic stem) (2). It is therefore believed that phototropins sense the fluence rate difference between the lit and shaded sides of the hypocotyl to initiate the phototropic response. Here we test this assumption directly by analyzing phototropism and early phototropin responses in a mutant with a strongly reduced light gradient across the hypocotyl. Our study identifies cell wall features that are essential to establish a light gradient across a translucid structure.

The authors are unaware of any potential conflict of interest

References

(1) Fankhauser & Christie (2015). Plant phototropic growth. Current Biology 25, R384-R389
(2) reuten, Hohm, Bergmann & Fankhauser (2013). Defining the site of light perception and initiation of phototropism in Arabidopsis. Current Biology 23, 1934-1938

> IL270. Invited Lecture

Symposium SENS-5 Blue Light and UV receptors. In Memoriam Winslow Briggs (John Christie)

PHOTOTROPIN-DEPENDENT SIGNALLING IN THE GREEN MICROALGA CHLAMYDOMONAS REINHARDTII

Authors: Yizhong Yuan[1], Marius Arend[2,3], Nooshin Omranian[2,3], Werth Emily[4], Aguila Ruiz-Sola[1], Angeliki Tsichla[1], Fabrizio Iacono[1], Leslie Hicks[4], Zoran Nikoloski[2,3], Dimitris Petroutsos[1]
Presenting Author: Dimitris Petroutsos
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In photosynthetic organisms, light is the energy source for photosynthesis to convert CO_2 into organic metabolites. However, whenever light is absorbed beyond the CO_2-assimilation capacity, excess energy becomes harmful causing generation of reaction oxygen species that can damage the photosynthetic apparatus and even lead to cell death. This is avoided by the activation of energy quenching (qE), a key photoprotective response that dissipates absorbed excitation energy as heat, ensuring cell survival even under adverse conditions. In Chlamydomonas, qE under excess light mainly requires the LHCSR3 protein. LHCSR3 is nucleus encoded and its expression is governed by transcriptional processes impacted, among others, by blue light perception via the photoreceptor phototropin (PHOT; Petroutsos et al., Nature, 2016).

Here we will present our latest data on comparative transcriptomics, proteomics and phosphoproteomics of low-light and high-light acclimated WT and PHOT deficient cells. We highlight possible players involved in the PHOT-dependent signaling cascade and also discuss the potential involvement of PHOT in other processes apart from adaptation to high light, i.e. in the regulation of carbohydrate metabolism.

> IL274. Invited Lecture

Symposium SENS-5 Blue Light and UV receptors. In Memoriam Winslow Briggs (John Christie)

RGS-LOV PHOTORECEPTORS

Authors: Brian Chow[1]
Presenting Author: Brian Chow
1) University of Pennsylvania

Summary

Our research group at the University of Pennsylvania discovers new photosensory proteins, engineers them as optogenetic tools, and applies these tools in mammalian synthetic biology studies to understand cell signaling dynamics. In this talk, we will present the bioinformatics discovery [1] and recent structure-function characterization [2] of a new class of fungal RGS-LOV photoreceptors that rapidly (~1 sec) localize to the plasma membrane through a blue light-switched, high-affinity, and direct electrostatic interaction with anionic membrane phospholipids. This finding is of significance because, to the best of our knowledge, natural photoreceptors have not been previously described to signal by direct association with membrane lipids. As optogenetic tools, these LOV proteins are widely applicable as single-component systems for dynamic membrane recruitment to control the signaling of fused proteins [3].

Conflicts of Interest

None

References

1. Glantz, S.T., Carpenter, E.J., Melkonian, M., Gardner, K.H., Boyden E.S., Wong, G.K-S., and Chow, B.Y. Functional and topological diversity of LOV domain photoreceptors, Proceedings of the National Academy of Sciences USA (2016) 113, E1442-E1451
2. Glantz, S.T., Berlew, E.E., Jayber, Z., Schuster, B.S., Gardner K.H., and Chow, B.Y., Directly light-regulated binding of RGS-LOV photoreceptors to anionic membrane phospholipids, Proceedings of the National Academy of Sciences USA (2018) 115 (33) E7720-E7727
3. Hannanta-anan, P., Glantz, S.T., and Chow, B.Y., Optically inducible membrane recruitment and signaling systems, Current Opinion in Structural Biology (2019) 57, 84-92

> IL271. Invited Lecture

Symposium SENS-5 Blue Light and UV receptors. In Memoriam Winslow Briggs (John Christie)

UV-B PHOTORECEPTOR SIGNALLING

Authors: Roman Ulm[1]
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Plants perceive UV-B radiation using the evolutionarily conserved UV Resistance Locus 8 (UVR8) photoreceptor. UVR8 is a homodimer in its ground state and monomerises upon UV-B absorption via specific intrinsic tryptophan residues. Active UVR8 monomers interact with the E3 ubiquitin ligase CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), initiating a molecular signalling pathway that results in gene expression changes. This signalling output leads to a broad range of UVR8-dependent physiological responses, including those that contribute to UV-B acclimation and stress tolerance. Regulation of the pathway is provided by the WD40-repeat proteins REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1) and RUP2, which facilitate UVR8 redimerization, disrupting the UVR8-COP1 interaction. I will present our latest understanding of how UVR8 activity is regulated upon UV-B photon absorption.

> IL269. Invited Lecture

Symposium SENS-5 Blue Light and UV receptors. In Memoriam Winslow Briggs (John Christie)

NATIVE MASS SPECTROMETRY REVEALS THE CONFORMATIONAL DIVERSITY OF THE UVR8 PHOTORECEPTOR

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In order to effect signal propagation, photoreceptor proteins undergo a wide variety of structural changes in response to light. These range from large-scale changes to domain architecture to more subtle motions of intrinsically-disordered regions (IDRs). Such changes (and indeed the very existence of IDRs) often present significant barriers to structural investigation using X-ray crystallography. Here, we have used native ion mobility mass spectrometry to enable – for the first time – interrogation of light-induced structural changes to a full-length photoreceptor protein (UVR8) in solution.[1]

UVR8 is a plant photoreceptor protein that regulates photomorphogenic and protective responses to UV light. UV-B light is absorbed by an unusual tryptophan chromophore in the inactive, homodimeric state, resulting in dissociation into monomers. Each monomer comprises an ostensibly well-folded β-propeller core domain and N and C-terminal tails, which are thought to be IDRs. Light-triggered monomerisation is required for activation of UVR8 and the C terminal IDR then facilitates functional binding to signalling partner COP1. To date, however, structural studies by X ray crystallography have been limited to the UVR8 core domains where the N and C-terminal IDRs have been truncated.[2-4]

By focussing pulses of UV-B light onto the ion source of an ion mobility mass spectrometer we were able to activate full-length UVR8 in its native state in solution. Our data reveal a high conformational diversity for both the UVR8 dimer and monomer. Strikingly, when the stabilising cross-dimer interactions are broken in the monomeric state, the N and C-terminal IDRs serve to destabilise the core fold resulting in highly extended conformations, which we argue are important for signalling. These data and their implications for the UVR8 signalling mechanism will be discussed in detail.

Our data demonstrate the power of native mass spectrometry to investigate the light-induced structural dynamics of photoreceptor proteins.

The authors declare no conflict of interest

References

1. Camacho, I. S. *et al.*, Native mass spectrometry reveals the conformational diversity of the UVR8 photoreceptor. *Proc. Natl. Acad. Sci. USA* **2019,** *116* (4), 1116-1125.
2. Christie, J. M. *et al.*, Plant UVR8 Photoreceptor Senses UV-B by Tryptophan-Mediated Disruption of Cross-Dimer Salt Bridges. *Science* **2012,** *335* (6075), 1492-6.
3. Wu, D. *et al.*, Structural Basis of Ultraviolet-B Perception by UVR8. *Nature* **2012,** *484* (7393), 214-9.
4. Zeng, X. *et al.*, Dynamic crystallography reveals early signalling events in ultraviolet photoreceptor UVR8. *Nature Plants* **2015,** *1*, 14006.

> IL276. Invited Lecture

Symposium SENS-5 Blue Light and UV receptors. In Memoriam Winslow Briggs (John Christie)

BES1 REGULATED BEE1 CONTROLS PHOTOPERIODIC FLOWERING DOWNSTREAM OF BLUE LIGHT SIGNALING PATHWAY IN ARABIDOPSIS

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BRI1-EMS-SUPPRESSOR 1 (BES1) functions as a key regulator in the brassinosteroid (BR) pathway that promotes plant growth. However, whether BES1 is involved in photoperiodic flowering is unknown. Here we report that BES1 acts as a positive regulator of photoperiodic flowering, but it cannot directly bind *Flowering Locus T (FT)* promoter. BR Enhanced Expression 1 (BEE1) is the direct target of BES1 and acts downstream of BES1. BEE1 is also a positive regulator of photoperiodic flowering. BEE1 binds directly to the *FT* chromatin to activate the transcription of *FT* and promote flowering initiation. More importantly, BEE1 promotes flowering in a blue light photoreceptor Cryptochrome 2 (CRY2) partially dependent manner, since it physically interacts with CRY2 under the blue light. Furthermore, BEE1 is regulated by both BRs and blue light. The transcription of BEE1 is induced by BRs, and the BEE1 protein is stabilized under the blue light. Our findings indicate that BEE1 is the integrator of BES1 and CRY2 mediating flowering, and BES1-BEE1-FT is a new signaling pathway in regulating photoperiodic flowering.

> P132. Poster

Symposium SENS-5 Blue Light and UV receptors. In Memoriam Winslow Briggs (John Christie)

THE ROLE OF PHOTOTROPIN INTERACTIONS IN ELICITING CHLOROPLAST MOVEMENTS

Authors: Justyna Łabuz[1], Paweł Hermanowicz[1,2], Agnieszka Katarzyna Banaś[2], Aneta Bażant[2], Olga Sztatelman[3],Wojciech Strzałka[2], John Christie[4], Halina Gabryś[2]
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Phototropins (phots) are plant blue/UVA light photoreceptors. They control intracellular movements and growth responses, enabling plants to fine-tune photosynthesis. Two phototropins, phot1 and phot2, have been identified in *Arabidopsis thaliana*. Their physiological functions overlap significantly, however sensitivity to light of elicited reactions depends on the phototropin[1]. In leaves, phototropins redundantly control chloroplast accumulation in weak blue light. Sustained chloroplast avoidance in strong blue light is triggered solely by phot2. Phot1 can elicit only a transient avoidance response. Previous observations of chloroplast movements in phototropin mutants indicate that interactions between phototropins enhance the avoidance response to pulses of high fluence blue light[2] and to continuous light[3]. In wild type plants, the accumulation response triggered by blue light pulses of different intensity does not depend on the duration of the pulse, only the dose is important. However, in the *phot2* mutant both the duration of the pulse and light intensity affect the response. Chloroplast accumulation in response to low-fluence light pulses is less sensitive in wild type plants than in the *phot2* mutant, suggesting that phot2 inhibits phot1 activity. To investigate the role of negative and positive phototropin interactions, photoreceptor dimerization and transphosphorylation have been examined. Yeast two hybrid assays show that different protein moieties are responsible for dimer formation in each phototropin[2]. Bimolecular fluorescence complementation assays suggest that those interactions occur *in planta*. Phototropin transphosphorylation has been examined using genetically modified kinases capable of utilizing bulky ATP analogs. Light-activated cross phosphorylation of phototropins has been assessed using phototropin kinase versions called Cerberus and GST-tagged phototropins with inactivated kinase domains (kinase dead - KD) as substrates. In accordance with literature[4], phototropin1-Cerberus transphosphorylases GST-tagged-phot1KD in light. However, transphosphorylation of GST-tagged-phot2KD by phototropin2-Cerberus has not been detected. Transphosphorylation of GST-tagged-phot1KD by phot2-Cerberus and GST-tagged-phot2KD by phototropin1-Cerberus seems to be induced by light. Thus, we hypothesize that phototropin transphosphorylation and dimerization is important for eliciting chloroplast movements, at least in non-saturating light conditions.

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References

1. Christie JM. 2007. Annual Review of Plant Biology 58, 21-45.
2. Sztatelman et al. 2016. Journal of Experimental Botany 67, 4963-4978.
3. Łabuz J et al. 2015. Plant Science 239, 238-249.
4. Schnabel J et al. 2018 The Journal of Biological Chemistry 293, 5613-5623.

> IL280. Invited Lecture

Symposium SENS-6 Flavin-Based Photofunctional Proteins. Evolution, Function and Applications (Aba Losi)

COMPARATIVE STUDIES OF LOV SWITCHING MECHANISMS AND CHARACTERISTICS

Authors: Igor Dikiy[1], Uthama Edupuganti[1,2], Zaynab Jaber[1,2], Matthew Cleere[1,3], Kevin Gardner[1,4,5]
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1) Structural Biology Initiative, CUNY Advanced Science Research Center 2) Ph.D. Program in Biochemistry, CUNY Graduate Center 3) Ph.D. Program in Biology, CUNY Graduate Center 4) Department of Chemistry & Biochemistry, City College of New York 5) Ph.D. Programs in Biochemistry, Biology, and Chemistry, CUNY Graduate Center

Introduction

Environmental cues regulate many biological processes, coordinating cellular pathways to respond to changing conditions. Such regulation is often initiated by sensory protein domains which expand their chemical repertoire by using small molecule ligands to convert environmentally-triggered changes into altered protein/protein interactions. Light-Oxygen-Voltage (LOV) domains present outstanding model systems for such processes, given their abilities to convert blue light into photochemically-driven formations of protein/flavin adducts and subsequent triggering of the allosteric control of effectors as diverse as kinases to DNA-binding domains. Combining biophysics, biochemistry and cell biology, we seek to gain insight into the mechanistic controls of such environmental sensing domains for both fundamental understanding and subsequent artificial control.

Methods

We combine approaches from: 1). biophysics, including NMR and EPR spectroscopies plus HDX-MS to monitor conformation and dynamics, 2). *In vitro* biochemistry studies of function, and 3). Cell-based examination of function in complex settings. Integrated data from these methods across LOV-HTH, LOV-HK and RGS-LOV-DUF settings will be discussed.

Results and Discussion

Of particular interest for us has been extending the current suite of high-resolution structures of dark state, inactive LOV proteins to challenging cases of defining spontaneously-activated “dark noise” and photoactivated conformations that are essential for a full understanding of control. This information has shown several commonalities of LOV signaling in diverse classes of sensory proteins, and further, develop structure-based mutations to shift these proteins among these states. In particular, we note substantial differences in the degree of conformational rearrangements observed among photoactivation among different LOV proteins. Further, we also observe a range of difference in “off” kinetics among proteins under study, often with substantial differences in the timing of adduct breakage and functional deactivation. We have taken advantage of this mechanistic understanding to develop the artificial regulation of such systems, both *in vitro* and in living cells.

Conclusions

Taken together, our work provides an integrated view of a fascinating class of natural switches and suggests routes by which these can be manipulated to achieve desired technological outcomes in a wide range of settings.

Acknowledgements

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Conflicts of Interest

None.

References

Dikiy *et al., Proc. Natl. Acad. Sci. USA* (2019); Glantz *et al., Proc. Natl. Acad. Sci. USA* **115**(2018): E7720; Losi *et al., Chem. Res.* **118**(2018): 10659; Motta-Mena *et al., Nat. Chem. Biol.* **10**(2014): 196.

> IL279. Invited Lecture

Symposium SENS-6 Flavin-Based Photofunctional Proteins. Evolution, Function and Applications (Aba Losi)

COMPUTATIONAL AND STRUCTURAL STUDIES OF LOV DOMAIN PHOTORECEPTORS REVEALS EVOLUTIONARY SELECTION OF A DIVERGENT SIGNALING PATHWAY

Authors: Brian Zoltowski[1]
Presenting Author: Brian Zoltowski
1) Southern Methodist University

Plants measure day-length and light-intensity to coordinate growth and development and reproduction with daily and seasonal changes in environmental conditions, however, the molecular details linking primary photochemistry to signal transduction remain incomplete. Recent research has indicated that divergent signaling mechanisms in two closely related Light-Oxygen-Voltage (LOV) domain containing proteins ZEITLUPE and FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 act in tandem to regulate the stability of circadian clock and photoperiodic flowering proteins to mediate daily and seasonal development.

Through a combination of computational and structural approaches we identify a key residue differentiating conformational changes in ZTL and FKF1. Further, we reveal that photon absorption results in global reorganization of a conserved dimer interface, leading to light-induced ordering of N-terminal and C-terminal signaling elements.

These results confirm a divergent mechanism within the ZTL family that differentiates these proteins from other members of the LOV superfamily and suggests that mechanisms of signal transduction in LOV proteins may be fluid across family members.

> IL277. Invited Lecture

Symposium SENS-6 Flavin-Based Photofunctional Proteins. Evolution, Function and Applications (Aba Losi)

LIGHT SIGNAL TRANSDUCTION THROUGH THE GLOBAL REGULATOR BLSA, A BLUF-TYPGE PHOTORECEPTOR FROM THE HUMAN PATHOGEN ACINETOBACTER BAUMANNII

Authors: Marisel Tuttobene[1], Estefanía Pavesi[1], María Alejandra Mussi[1]
Presenting Author: María Alejandra Mussi
1) Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI- CONICET). Argentina.

Light exerts a global effect on the physiology of the important human pathogen *Acinetobacter baumannii* at moderate temperatures, likely modulating the microorganism\' persistence in the environment. Persistence in the clinical settings is a key aspect determining *A. baumannii* success as a pathogen. We have shown that light modulates biofilm formation, motility and virulence against *Candida albicans*. Light also modulates metabolic pathways including trehalose biosynthesis, a disaccharide likely involved in resistance to diseccation, and the phenylacetic acid degradation pathway. Light enhances antioxidant enzyme levels such as catalase, and modulates susceptibility and tolerance to some antibiotics. In addition, light induces the expression of whole gene clusters and pathways, including one involved in lipid modification, the complete type VI secretion system (T6SS) and efflux pumps. Many of these processes are controlled by a short Blue Light Using Flavin (BLUF) protein, BlsA, the only canonical photoreceptor codified in the genome of *A. baumannii*. We have disclosed the light signal transduction mechanism mediated by this photoreceptor by showing that BlsA binds to and antagonizes the functioning of the transcriptional repressor Fur only in the dark at 23ºC, likely by reducing its ability to bind to the siderophore Acinetobactin promoters, with enhanced gene expression of the corresponding genes and growth under iron deprivation at this condition. Recently, we have broadened our understanding of BlsA functioning by showing that this photoreceptor can antagonize the functioning of other transcriptional regulators also under blue light such as the acetoin repressor AcoN. Indeed, BlsA interacts with AcoN only under blue light but not in the dark at 23 °C. Moreover, the acetoin catabolic genes *acoA*, acoB and *acoC* were induced at this condition in a BlsA and AcoN-dependent manner. Consistently, growth on acetoin was supported under blue light rather than in the dark through BlsA and AcoN. The data support a model in which BlsA interacts with and likely sequesters the acetoin repressor in the presence of light, relieving acetoin catabolic genes from repression and leading to much better growth at this condition. The phenomena depend on temperature, consistently with recent findings indicating BlsA functioning only at low-moderate temperatures. Overall, the global regulator BlsA can function both under blue light and in the dark modulating different transcriptional regulators simultaneously at moderate temperatures, leading to regulation of different sets of genes and cellular processes. BlsA probes to be unique regarding its dual activity under illumination and in the dark.

The authors declare no conflicts of interest.

> IL282. Invited Lecture

Symposium SENS-6 Flavin-Based Photofunctional Proteins. Evolution, Function and Applications (Aba Losi)

BLUF DOMAIN STRUCTURAL DYNAMICS PROBED BY THE ULTRAFAST TRANSIENT INFRARED RESPONSE OF THE NONCANONICAL AMINO ACID AZIDOPHENYLALANINE

Authors: Christopher R. Hall[§†], Jinnette Tolentino[‡], James Iuliano[‡], Katrin Adamczyk[§], Andras Lukacs[#], Gregory M. Greetham[‖], Igor Sazanovich[‖], Peter J. Tonge[‡*] and Stephen R. Meech[§*]
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The BLUF (blue light using flavin) domain proteins are an important class of blue light sensing flavoproteins that have recently found applications in optogenetics. Compared to other photoreceptor flavoproteins both the primary photochemical mechanism and the structure change between light- and dark-adapted states is poorly characterised. Uniquely the light and dark activated state have the flavin chromophore in the same chemical structure and oxidation state, and the indication of photoactivation is simply a 15 nm red shift. This has led to numerous experimental and theoretical investigations of the primary photochemical process and subsequent structure changes in the protein leading to formation of the signalling state.

Here we probe the mechanism of photactivation through incorporation of the noncanonical amino acid (ncAA) azidophenylalanine (AzPhe) at two key positions in the H-bonding environment of the isoalloxazine chromophore. This is done for two different BLUF domains, PixD and $AppA_{BLUF}$, which exhibit quite different photokinetics. Both proteins retain their photoactivity after substitution. We employ steady state and ultrafast time resolved infrared difference measurements of the azido mode to extract site-specific information on light driven structure changes following optical excitation of the isoalloxazine chromophore. The AzPhe dynamics are shown to be an effective probe of BLUF domain photoactivation. The data reveal significant differences between the two BLUF domains, and a differential response of the two sites to optical excitation of the chromophore. The results will be discussed in terms of evolution in the H-bond structure between dark and light adapted states. Other applications of ncAA substitution to probe photosensor photodynamics will be addressed.

> IL283. Invited Lecture

Symposium SENS-6 Flavin-Based Photofunctional Proteins. Evolution, Function and Applications (Aba Losi)

CONTROLLING NUCLEIC ACIDS BY LIGHT

Authors: Andreas Möglich[1]
Presenting Author: Andreas Möglich
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Sensory photoreceptors underpin diverse adaptations of organismal behavior, lifestyle and physiology to incident light. In optogenetics, photoreceptors double as genetically encoded, light-gated actuators and enable the noninvasive control of cellular circuits with spatiotemporal precision. Against this backdrop, we investigate and engineer blue-light-responsive receptors of the light-oxygen-voltage (LOV) family that mediate optogenetic control of various nucleic-acid-based processes, e.g., transcription, translation and endonuclease activity. Biochemical analyses of receptor structure, function and signaling mechanism unravel the molecular bases for light-dependent allostery and inform additional protein engineering efforts.

> IL281. Invited Lecture

Symposium SENS-6 Flavin-Based Photofunctional Proteins. Evolution, Function and Applications (Aba Losi)

FLAVIN-BINDING FLUORESCENT PROTEINS AS GENETICALLY-ENCODED SINGLET OXYGEN PHOTOSENSITIZERS

Authors: Joaquim Torra[1,2], Rubén Ruiz-González[1], Santi Nonell[1]
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Rational engineering of natural blue-light photoreceptors has provided a new class of small proteins with a flavin mononucleotide (FMN) chromophore, known as flavin-binding fluorescent proteins (FbFPs). These proteins exhibit bright green fluorescence and are capable of photosensitizing singlet oxygen ($^1O_2$) and other reactive oxygen species upon blue light illumination. While their fluorescent properties have been largely exploited, their photosensitization ability and phototoxicity have remained less explored. In this talk, the photophysical, photosensitizing and antimicrobial properties of eleven FbFPs derived from different organisms will be discussed [1]. In particular, special attention will be given to miniSOG, the first FbFP developed for $^1O_2$ generation, for which we have recently solved its high-resolution crystal structure and unveiled the phototransformations that it undergoes upon exposure to blue light [2]. The combination of structural and spectroscopic studies has provided a robust framework to explain its complex photophysics and to help reconcile the modest $^1O_2$ generation and its excellent performance in photo-oxidation experiments. Overall, our results are relevant to provide a rational basis for guiding the evolution of FbFPs towards desired photophysical attributes and contribute to expanding the toolbox of FbFPs as genetically encoded photosensitizers.

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References

[1] Endres, S.; Wingen, M.; Torra, J.; Ruiz-González, R.; Polen, T.; Bosio, G.; Bitzenhofer, N.; Hilgers, F.; Gensch, T.; Nonell, S.; Jaeger, K-E.; Drepper, T.; An optogenetic toolbox of LOV-based photosensitizers for light-driven killing of bacteria, *Sci. Rep*, **2018**, 8, 1-14
[2] Torra, J.; Lafaye, C.; Signor, L.; Aumonier, S.; Flors, C.; Shu, X., Nonell, S.; Gotthard, G.; Royant, A.; Tailing miniSOG: Structural Bases of the Complex Photophysics of a Flavin-Binding Singlet Oxygen Photosensitizing Protein. *Sci. Rep*, **2019**, 9, 1-10

> IL278. Invited Lecture

Symposium SENS-6 Flavin-Based Photofunctional Proteins. Evolution, Function and Applications (Aba Losi)

BLUE LIGHT PHOTORECEPTORS FROM PLANT SYMBIOTIC BACTERIA

Authors: Aba Losi[1], Eleonora Consiglieri[1]
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In recent years novel and largely unforeseen biological photoreceptors have been discovered in many bacteria, in most cases with poorly understood in vivo functions. Bacterial photoreceptors mainly belong to two superfamilies: blue light (BL) sensing LOV proteins and red/far red (R/FR) light sensing bacteriophytochromes (BphP), binding respectively flavin mononucleotide (FMN) and biliverdin-IXa (BV) as chromophores. LOV proteins and phytochromes are also the main photoreceptors of plants, and it is clear that many bacteria that are plant pathogens or symbionts are able to detect the same colors as their natural host. In this paper we will present the biophysical characterization of novel BL receptors from *Methylobacterium radiotolerans*, a radiation resistant, nitrogen fixing bacterium, able to promote plant growth and grow facultatively on methanol (2). In addition, *M. radiotolerans* is an opportunistic human pathogen and has a high potential for being employed in soil bioremediation. As other *Methylobacteria*, *M. radiotolerans* bears genes for several BphP and BL receptors, that we started to investigate during the last months by means of steady state and time-resolved spectroscopy. In particular we focus here on a LOV protein that show high structural stability and an extremely long photocycle in its wild type form. Sequence analysis revealed some peculiarities with respect to the majority of LOV domains; point mutations evidenced that this *M. radiotolerans* LOV photoreceptor is a promising candidate for biophysical applications, chiefly as fluorescent reporter and as genetically encoded photosensitizer (3). The possible roles of photoreceptors in the physiology of *M. radiotolerans* is also discussed, on the basis of bioinformatics analysis.

References

(1) Losi, Aba, and Wolfgang Gärtner. "Solving Blue Light Riddles: New Lessons from Flavin-binding LOV Photoreceptors." *Photochemistry and photobiology* 93.1 (2017): 141-158.
(2) Thulasi, Kavitha, et al. "Efficient methanol-degrading aerobic bacteria isolated from a wetland ecosystem."*Archives of microbiology*" (2018): 829-833.
(3) Westberg, Michael, et al. "Temperature sensitive singlet oxygen photosensitization by LOV-derived fluorescent flavoproteins." *The Journal of Physical Chemistry B* 121.12 (2017): 2561-2574.

> OC108. Oral Communication

Symposium SENS-6 Flavin-Based Photofunctional Proteins. Evolution, Function and Applications (Aba Losi)

BIOLUMINESCENCE DRIVEN CONTROL OF PHOTOSENSORY PROTEINS

Authors: Emmanuel Crespo[1], Gerald Lambert [2], Nathan Shaner[2], Ute Hochgeschwender[1]
Presenting Author: Emmanuel Crespo
1) Central Michigan University CMU 2) UC San Diego UCSD

Bioluminescence is light emitted by a luciferase oxidizing its substrate. We previously demonstrated that such "biological" light can activate optogenetic elements, such as channelrhodopsins and pumps, effecting membrane potential changes and resulting in activation or silencing of neurons in vitro and in vivo [1,2]. We explored whether bioluminescent light production can be utilized beyond activating ion-moving photoreceptors to the larger array of photosensory proteins employed as optical switches in cellular processes such as protein translocation and transcription [3].

In initial proof-of-concept experiments we co-transfected HEK293 cells with a blue light emitting luciferase and a blue light sensing photoreceptor. Light emitters were sbGLuc, a copepod luciferase variant, NanoLuc, a luciferase derived from shrimp, as well as two novel engineered synthetic luciferases. Photoreceptors were CRY/CIB, a light-gated dimerization system [4], and eLOV, based on light dependent protein unhinging [5]. Bioluminescence driven activation of these photoreceptors was measured as increased transcription of luminescent and fluorescent reporter proteins in direct comparison to LED driven activation.

Quantification of bioluminescence driven photoreceptor activation revealed that both light-gated switches, cryptochrome protein dimerization and light-oxygen-voltage J-alpha helix unfolding can be efficiently activated by biological light sources. Furthermore, the higher light emission of our synthetic luciferases resulted in better activation of transcription.

There are many ways to improve further on these basic results. Collectively, bioluminescence driven activation of the larger families of photoreceptors will expand their use for in vivo applications that benefit from non-invasive light sources and engagement of spatially distributed cells.

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The authors declare no conflicts of interest.

References

1. Berglund, K., Birkner, E., Augustine, G. J. & Hochgeschwender, U. Light-Emitting Channelrhodopsins for Combined Optogenetic and Chemical-Genetic Control of Neurons. *PLoS One* **8,** e59759 (2013).
2. Berglund, K. *et al.* Luminopsins integrate opto- and chemogenetics by using physical and biological light sources for opsin activation. *Proc. Natl. Acad. Sci.* **113,** 201510899 (2016).
3. Losi, A., Gardner, K. H. & Moglich, A. Blue-Light Receptors for Optogenetics. *Chem. Rev.* **118,** 10659–10709 (2018).
4. Liu, Q. & Tucker, C. L. Engineering genetically-encoded tools for optogenetic control of protein activity. *Curr. Opin. Chem. Biol.* **40,** 17–23 (2017).
5. Wang, W. *et al.* A light- and calcium-gated transcription factor for imaging and manipulating activated neurons. *Nat. Biotechnol.* **35,** 864–871 (2017).

> OC109. Oral Communication

Symposium SENS-6 Flavin-Based Photofunctional Proteins. Evolution, Function and Applications (Aba Losi)

INSIGHTS INTO SIGNAL TRANSDUCTION IN LIGHT-OXYGEN-VOLTAGE (LOV) RECEPTORS

Authors: Julia Dietler[1], Renate Gelfert[1], Jennifer Kaiser[1], Andreas Möglich[1]
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Light-oxygen-voltage (LOV) domains are capable of sensing blue light as an environmental stimulus and converting it into a biochemical signal in a wide variety of proteins. While the initial photochemistry is highly conserved and well-investigated, the allosteric mechanism by which formation of the cysteinyl-flavin adduct leads downstream signaling is not sufficiently clarified by now. As allostery of signal transduction can vary greatly in LOV domains, e.g. Jα helix unfolding to release its effector in *Avena sativa* phototropin 1 LOV2[1] or Jα rotation in *Bacillus subtilis* YtvA[2–4], no decisive mechanism of how those diverse outputs can be obtained is known. In general, it is hypothesized that protonation of N5 of the flavin cofactor leads to a change in polarity and is sufficient to induce a “flip” of the side chain of a conserved glutamine which in turn is required for signal transduction[5–7]. In this study, we further investigate LOV domain activation and specifically signal transduction in *Avena sativa* phototropin 1 LOV2, the engineered LOV receptor YF1 and the natural LOV receptor PAL. By analyzing the competence of downstream signaling of distinct variants we try to get new insights into signal transduction in LOV receptors.

References

[1] S. M. Harper, L. C. Neil, K. H. Gardner, *Science* **2003**, *301*, 1541–1544.
[2] O. Berntsson, R. P. Diensthuber, M. R. Panman, A. Björling, A. J. Hughes, L. Henry, S. Niebling, G. Newby, M. Liebi, A. Menzel, et al., *Struct. (London, Engl. 1993) Press* **2017**, 933–938.
[3] C. Engelhard, R. P. Diensthuber, A. Möglich, R. Bittl, *Sci. reports, Press* **2017**, 1–10.
[4] O. Berntsson, R. P. Diensthuber, M. R. Panman, S. Niebling, H. Takala, J. A. Ihalainen, S. Kerruth, J. Heberle, M. Liebi, R. Henning, et al., *Nat. Commun.* **2017**, 1–7.
[5] A. I. Nash, W. Ko, S. M. Harper, K. H. Gardner, **2008**, 13842–13849.
[6] E. F. Yee, R. P. Diensthuber, A. T. Vaidya, P. P. Borbat, C. Engelhard, J. H. Freed, R. Bittl, A. Möglich, B. R. Crane, *Nat. Commun.* **2015**, *6*, 10079.
[7] M. A. Jones, K. A. Feeney, S. M. Kelly, J. M. Christie, *J. Biol. Chem.* **2007**, *282*, 6405–6414.

> P133. Poster

Symposium SENS-6 Flavin-Based Photofunctional Proteins. Evolution, Function and Applications (Aba Losi)

CHARACTERIZATION OF A SINGLET OXYGEN PHOTOSENSITIZING PROTEIN IN A MAMMALIAN CELL LINE

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There has been a great effort over the last 10 years to develop an optogenetic actuator that can selectively and efficiently generate singlet molecular oxygen, $O_2(a^1\Delta_g)$.[1] This would be a valuable tool for mechanistic studies of oxidative stress and eustress, complementing optogenetic actuators that produce the superoxide radical anion, for example.[1]

Several variations of **S**inglet **O**xygen **P**hotosensitizing **P**roteins (SOPPs), in which protein-bound Flavin Mononucleotide is the $O_2(a^1\Delta_g)$ sensitizer, have been developed.[2] Among these, SOPP3, selectively makes $O_2(a^1\Delta_g)$ at the expense of reactions that produce superoxide.[2] A key part of this work was the detailed characterization of SOPP3 photophysics in solution.

We have now successfully incorporated SOPP3 into Flp-In™ T-rex™ 293 cells via stable transfection. In our procedure, we achieved selective site-dependent placement of SOPP3 in the outer mitochondrial membrane and plasma membrane by fusion to TOMM20 and Lck peptides, respectively. We have examined the response of these cells to the irradiation of SOPP3 with blue light, covering a wide range of incident light intensities (*i.e.*, a wide range of the $O_2(a^1\Delta_g)$ dose). Our results confirm that, when localized in a cell, SOPP3 can be a useful mechanistic tool in studies of $O_2(a^1\Delta_g)$-mediated oxidative stress and eustress.

References

(1) Trewin, A. J.; Berry, B. J.; Wei, A. Y.; Bahr, L. L.; Foster, T. H. ; Wojtovich, A. P. Light-Induced Oxidant Production by Fluorescent Proteins. *Free Rad. Biol. Med.* **2018**, *128*, 157-164.
(2) Westberg, M.; Bregnhøj, M.; Etzerodt, M. ; Ogilby, P. R. No Photon Wasted: An Efficient and Selective Singlet Oxygen Photosensitizing Protein. *J. Phys. Chem. B* **2017**, *121*, 9366-9371.

> P134. Poster

Symposium SENS-6 Flavin-Based Photofunctional Proteins. Evolution, Function and Applications (Aba Losi)

TOWARDS ENGINEERING OF A SPLIT FLAVIN-BASED FLUORESCENT PROTEIN

Authors: Anna Yudenko[1], Anastasia Smolentseva[1], Vera V. Nazarenko[1], Ivan M. Goncharov[1], Alina Remeeva[1], Ivan Gushchin[1]
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Introduction

In the recent years, LOV domains of several photoreceptor proteins have been developed into flavin-based fluorescent proteins (FbFPs). Under some circumstances, FbFPs can outperform commonly used beta-barrel fluorescent proteins such as GFP. Here, we show that CagFbFP, a small thermostable FbFP based on a protein from *Chloroflexus aggregans* (Nazarenko et al., 2019), can potentially be engineered into a split protein.

Methods

We used bioinformatics to identify the loops with variable length among the known LOV domains, which are the sites where the protein might tolerate cutting into two fragments. Using genetic engineering, we inserted poly-Gly/Ser fragments into the identified sites, and checked the folding and thermostability of the resulting constructs. We also cloned the fragments into separate plasmids and checked the fluorescence of the co-expressed fragments *in vivo*.

Results and Discussion

We have identified three positions where poly-Gly/Ser insetions are tolerated, as CagFbFP remains stable and fluorescent after introduction of flexible fragments into these loops. Therefore, the identified sites could potentially tolerate splitting or insertion of a sensor domain. Co-expression of the split fragments, fused to interacting coiled coils, in *Escherichia coli* resulted in appearance of characteristic fluorescence. Thus, we conclude that CagFbFP can serve as a basis for engineering of a split flavin-based fluorescent protein. Using a split FbFP might be advantageous in anaerobic conditions or when fast and reversible association of the split fragments is required.

Acknowledgements

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Conflicts of Interest

There are no conflicts to declare.

References

Nazarenko, V.V., Remeeva, A., Yudenko, A., Kovalev, K., Dubenko, A., Goncharov, I.M., Kuzmichev, P., Rogachev, A.V., Buslaev, P., Borshchevskiy, V., et al. (2019). A thermostable flavin-based fluorescent protein from *Chloroflexus aggregans*: a framework for ultra-high resolution structural studies. Photochem. Photobiol. Sci.

> P135. Poster

Symposium SENS-6 Flavin-Based Photofunctional Proteins. Evolution, Function and Applications (Aba Losi)

INTERPLAY BETWEEN THE "FLIPPING" GLUTAMINE, A CONSERVED PHENYLALANINE, WATER AND HYDROGEN BONDS IN THE CHROMOPHORE CAVITY OF A BLUE-LIGHT SENSING LOV DOMAIN

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This work combines time-resolved photoacoustics (PA) and molecular dynamics (MD) simulations to investigate a conserved phenylalanine residue within the LOV (Light, Oxygen, Voltage) photosensing domain of blue-light (BL) photoreceptors. LOV domains bind in most cases flavin mononucleotide (FMN) as chromophore. BL triggers the reversible formation of a photoproduct (LOV_{390}) where FMN(N5) is protonated and FMN(C4a) becomes covalently bound to a cysteine. LOV_{390} thermally returns to the parent state (LOV_{447}) with lifetime τ_{rec} between few seconds and many hours; τ_{rec} is affected by many factors, e.g. the hydrogen bond (HB) network around FMN, hydration, steric effects, extent of light-induced conformational changes, energy content of LOV_{390}.

In the LOV domain of wild type (wt) YtvA from Bacillus subtilis F46 is one of the few residues undergoing a prominent light-driven conformational change. For the mutated F46A and F46Y the photocycle is strongly accelerated, light-induced structural changes are smaller and F46Y- LOV_{390} has lower energy content (80 vs 160 kJ/mol). Four independent MD simulations for each variant of LOV_{447} and LOV_{390} reveal an overall very stable structure of YtvA-LOV. The largest variations emerge for the HB network that include FMN, Q123, N104 and N94. HB with N104 and N94 are fixed, but Q123 has a larger flexibility and in wt-LOV_{447} can adopt two alternative conformations. Q123 movements act in concert with the flexibility of F46 and with slight shifts of FMN. In LOV_{390}Q123 is much more rigid, strictly remaining in the orientation adopted in the crystal. In LOV_{447}, however, Q123 is able to flip in a LOV_{390}-like conformation, in particular when water enters the binding site. Water molecules cannot enter and escape easily from the binding cavity: however, when present, water mediates/constrains the conformations of Q123.

In F46A the wider binding cavity allows more space for Q123 sidechain. In LOV_{447} Q123 forms an HB with FMN (O4). Interestingly, Q123 is locked in this LOV_{447}-like conformation also in the photoproduct, that could be the cause of the fast photocycle observed.

In LOV_{447} of F46Y the hydroxyl group of Y46 fixes a water molecule, which then induces a Q123 conformation similar to wt-LOV_{390}: this pseudo-photoproduct conformation may account for the faster photocycle observed. The same Q123 orientation is adopted in LOV_{390} of F46Y, but not mediated by water. The HB network involving FMN, N94 and N104 is in this case disturbed, in agreement with the lower photostability of this mutant.

> IL288. Invited Lecture

Symposium SENS-7 The Phytochrome Diversity: Mechanisms Light Signal Integration and Applications in Optogenetics (Wolfgang Gärtner)

WHY AND HOW ARE PHYTOCHROMES KNOTTED?

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Members of the phytochrome family of photoreceptors contain a deep figure-of-eight knot. While the past decade has seen remarkable progress in understanding the photochemistry, dynamic structural properties, and signaling pathways of phytochromes, very little is known about the function of the knot or the folding pathway to form this complex topology. With respect to knot function, we have hypothesized that signal transduction by phytochromes is enabled by their unusual topology. Absorption of a 700 nm photon by the chromophore directs 41 kcal/mol into the protein; the knot may rigidify the photosensory core of phytochrome so that work is done to reposition the effector domain appropriately in the transition from dark to lit state, rather than permitting energy losses to random motions. To test this model, we are studying the biochemistry and structural biology of the signal transduction pathway of the bacterial phytochromes of *Deinococcus radiodurans*and *Ramlibacter tatouinensis*. We have designed knotless bacterial phytochromes and will test folding, photochemistry and the light-dependence of signaling with the combined applications of a colorimetric in-cell assay for functional signaling (Etzl *et al*., 2018) with *in vitro*photochemical and enzymatic assays (Baker *et al*., 2018). The second intriguing aspect of the knotted topology of phytochrome is knot formation during the folding process. The ability of *E. coli* (a species that does not normally produce phytochrome) to produce numerous phytochromes from different plant and microbial species implies no dedicated phytochrome-specific chaperone is required. It seems likely, however, that widely distributed non-specific chaperones and/or the ribosome itself (Dabrowski-Tumanski*et al*., 2018) play a central role in phytochrome folding. We are thus addressing the contribution of the ribosome-associated chaperone Trigger Factor *via*in-cell steady state and *in vitro*kinetic experiments. Work in the field of knotted protein folding is rapidly gaining traction as single molecule techniques and computational folding simulations advance. Phytochrome is an excellent model system for understanding both the why and the how of knotted protein structures.

References

Etzl, Lindner, Nelson, and Winkler, J Biol Chem **293**:9078-9089, 2018.
Baker, Satyshur, Moreno Morales, and Forest, J Bacteriol **198**:1218-1229, 2018.
Dabrowski-Tumanski, Piejko, Niewieczerzal, Stasiak, and Sulkowska, J Phys Chem B **122**:11616-11625, 2018.

> IL287. Invited Lecture

Symposium SENS-7 The Phytochrome Diversity: Mechanisms Light Signal Integration and Applications in Optogenetics (Wolfgang Gärtner)

MOLECULAR DETERMINANTS OF ASYMMETRIC PHYTOCHROME ACTIVATION AND SIGNALING

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Phytochromes constitute a diverse family of photoreceptors with remarkably different domain architectures[1] of their sensory module as well as a variety of covalently linked effector domains. One subfamily corresponds to bacteriophytochromes that predominantly absorb red light thereby being converted to a far-red absorbing state. Conformational changes induced by this transition regulate downstream effector domains or influence protein-protein interactions. Both of these properties have been successfully employed in the generation of optogenetic tools.

To improve our understanding of molecular mechanisms involved in allosteric regulation of enzymatic effector domains upon red light activation, we focus on the family of phytochrome activated diguanylate cyclases (PadCs). Using various tools of structural biology, we have obtained evidence for an asymmetric activation mechanism, realized by different conformational states of the two protomers within the parallel dimer of full-length PadCs[2]. These observations differ from bathyphytochromes or other canonical phytochromes that can stabilize symmetrically activated homodimers[3]. To identify molecular determinants of destabilizing two activated protomers in PadCs, we recently combined the biochemical characterization of chimeras of different naturally occurring PadCs[4] with site-directed mutagenesis efforts targeting the stability of the dimer interface. New insights will be presented in the context of the structural characterization[2,5] of one representative PadC member. The results not only confirm the involvement of the dimer interface in influencing the biliverdin cofactor environment, but also that symmetric activation is possible by reducing the stability of the PadC dimer interface. However, a fine balance between interface stability and asymmetric activation is required for light regulation of the diguanylate cyclase output.

All in all, the evolutionary playground of the dimer interface has resulted in remarkably different modes of signal integration and allosteric regulation in diverse phytochrome-coupled systems. Different phytochrome sensors are likely to show contrasting effects in engineering approaches of novel optogenetic systems. Rational approaches might therefore appear more challenging, but at the same time, chances of finding functional phytochrome-effector couples with beneficial novel properties can be enhanced by screening the natural diversity of red light photoreceptors.

References

1 Gourinchas G, Etzl S and Winkler A. (2019) *Curr Opin Struct Biol*, **in press**.
2 Gourinchas G, Heintz U and Winkler A. (2018) *eLife*, **7**, e34815. doi: 10.7554/eLife.34815
3 Takala et al. (2014) *Nature*, **509**, 245-8. doi: 10.1038/nature13310
4 Gourinchas G, Vide U and Winkler A. (2019) *J Biol Chem*, **294**. doi: 10.1074/jbc.RA118.007260
5 Gourinchas G *et al*. (2017) *Sci Adv*, **3**, e1602498. doi: 10.1126/sciadv.1602498

> IL290. Invited Lecture

Symposium SENS-7 The Phytochrome Diversity: Mechanisms Light Signal Integration and Applications in Optogenetics (Wolfgang Gärtner)

SPECTROSCOPIC INVESTIGATIONS ON THE PHOTOCONVERSION OF BACTERIOPHYTOCHROME FROM PICOSECONDS TO MILLISECONDS

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In phytochrome proteins light absorption causes isomerization of the biliverdin chromophore, which triggers a series of structural changes to activate the signalling domains of the protein. This process takes place in multiple time-scales and length-scales, from picoseconds to milliseconds, and from Å-scale to several nanometer-scale, respectively. Although the general atomic structures of the resting states (Pr and Pfr-states) of the photosensory units are known, the structural changes along the reaction chain are elusive. Therefore, the molecular mechanism of signal transduction remains to be solved. In our studies, we utilize several spectroscopic techniques, such as ultra-fast transient Vis-absorption and IR-spectroscopy as well as slower time-scale experiments, such as flash-photolysis and step-scan FTIR spectroscopy, to reveal the dynamic picture of the signal transduction of bacteriophytochromes. We will show, for example, how the coordinated water molecules nearby the chromophore play a crucial role in stabilizing the Pr and Pfr states. Further, we show how the hydrogen bonds to the biliverdin D-ring carbonyl become disordered in the first intermediate (Lumi-R) forming a dynamic microenvironment, then completely detach in the second intermediate (Meta-R), and finally reform in the signaling state (Pfr). Additional changes in the protein backbone are detected already within microseconds in Lumi-R. We have also focused on the roles of the amino acids nearby the biliverdin molecule and how they are playing in preserving the chemical properties of bilin in the resting Pr-state. By using pH-dependent UV-Vis spectroscopy and spectral decomposition modeling, we confirm the importance of H260 for biliverdin protonation. Further, we demonstrate that in the canonical bacteriophytochromes, the pKa value of the phenol group of the Y263 is uncommonly low.

This directly influences the protonation of the bilin molecule and likely the functional properties of the protein. Our studies rationalize the chromophore environment in the resting states but also how the isomerization process is linked to the global structural rearrangement in the sensory receptor.

References

Nils Lenngren, Petra Edlund, Heikki Takala, Brigitte Stucki-Buchli, Jessica Rumfeldt, Ivan Peshev, Heikki Häkkänen, Sebastian Westenhoff, and Janne A. Ihalainen: Coordination of the Biliverdin D-ring in Bacteriophytochromes. Phys. Chem. Chem. Phys. 2018, 20, 18216-18225.

Janne A. Ihalainen, Emil Gustavsson, Lea Schroeder, Serena Donnini, Heli Lehtivuori, Linnéa Isaksson, Christian Thöing, Vaibhav Modi,Oskar Berntsson, Brigitte Stucki-Buchli, Alli Liukkonen, Heikki Häkkänen, Elina Kalenius,Sebastian Westenhoff, and Tilman Kottke: Chromophore−Protein Interplay during the Phytochrome Photocycle Revealed by Step-Scan FTIR Spectroscopy, JACS 2018, 140, 12396-12404.

Jessica A. Rumfeldt, Heikki Takala, Alli Liukkonen and Janne A. Ihalainen: UV-Vis Spectroscopy Reveals a Correlation Between Y263 and BV Protonation States in Bacteriophytochromes, Photochemistry and Photobiology, 2019 In Press.

> IL289. Invited Lecture

Symposium SENS-7 The Phytochrome Diversity: Mechanisms Light Signal Integration and Applications in Optogenetics (Wolfgang Gärtner)

TIME-RESOLVED SERIAL FEMTOSECOND CRYSTALLOGRAPHY OF PHYTOCHROMES

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A remaining grand challenge in structural biology is to capture atomic resolution structures in time-resolved snapshots to create "movies" of macromolecules engaged in function. Fortunately, we are experiencing step-changes in time-resolved functional studies that are linked to serial femtosecond crystallography (SFX). This room temperature technique exploits micron-size crystals and fs pulses from X-ray Free Electron Lasers (XFELs) to yield high quality crystallographic and spectroscopic results from macromolecules in their ground state, or at nearly any timepoint after perturbation(s).

To these ends, our collaboration has developed on-demand acoustic tape drive methods to deliver individual nL - pL size droplets of a microcrystal slurry into the interaction region at the LCLS and at SACLA [1,2,3]. The distance between the acoustic injector and the interaction region is used to perturb the samples with visible light pulses *e.g.* pump-probe time-resolved SFX. We used these strategies to collect time-resolved SFX data to as high as 1.5 Å resolution, from various constructs of three different light-sensitive phytochromes (Dr-BphP, Te-PixJ, At-Agp2), using a variety of illumination schemes (from blue light to far-red illumination with pump times ranging from ns to ms) and delay times ranging from ps through hundreds of seconds.

Dr-BphP and At-Agp2 phytochromes act as red/far-red photo-switches that are activated/deactivated by absorption of red or far-red light, respectively, by means of a covalently bound bilin chromophore. Using a similar bilin cofactor but different protein-ligand covalent linkages, Te-PixJ photo-switches in response to blue/green light. The structural basis for how these proteins function is still poorly understood and our emerging time-resolved SFX results will be presented.

Acknowledgements

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References

[1] Kern, J., *et al* (2018) *Nature 563*, 421-425
[2] Fuller, F. D., *et al* (2017) *Nature Methods 14*, 443-449
[3] Roessler, C. G., *et al* (2016) *Structure* 24, 631-640

> IL286. Invited Lecture

Symposium SENS-7 The Phytochrome Diversity: Mechanisms Light Signal Integration and Applications in Optogenetics (Wolfgang Gärtner)

CONVERTING FAR-RED PHYCOBILIPROTEINS INTO MUCH BRIGHTER NEAR-INFRARED FLUORESCENT PROTEINS

Authors: Jian-Yun Hou[1], Zi-Zhu Tan[1], Ya-Nan Hou[1], Xiao-Dan Li[1], Bao-Qing Zhao[1], Ming Zhou[1], Astrid Höppner[2], Wolfgang Gaertner[3], Kai-Hong Zhao[1]
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Photosynthesis relies on energy transfer from light-harvesting complexes to reaction centers. Energy transfer in cyanobacteria and some phylogenetically related organisms proceeds from hundreds of chromophores located in the peripheral antenna, the phycobilisomes (PBSs), to the reaction centers in the membrane. In the PBS cores, phycocyanobilin (PCB) chromophores are covalently attached to apo-allophycocyanins (ApcA, ApcB, ApcD, and ApcF). In an ApcF2 from a far-red acclimated cyanobacterium *Chroococcidiopsis thermalis* sp. PCC7203, PCB is non-covalently bound with ApcF2, so extremely red-shifted in its absorption maximum (I_{Amax} ~ 675 nm). Far-red (FR) and near-infrared (NIR) emitting chromophores extend the application of fluorescent proteins (FPs) into the region of maximal transmission for most tissues. We have molecularly evolved ApcF2 into a set of FR FPs termed BDFP1.2, 1.3, and 1.6 (I_{Fmax} ~ 665 nm), and a set of NIR FPs termed BDFP1.1, 1.4, and 1.5 (I_{Fmax} ~ 705 nm). These BDFPs are covalently bound with biliverdin (BV) chromophore. After determing the crystal struture of one FR BDFP, it is verified that in the FR BDFP, the sulfhydryl group of Cys72 is added at $C3^2$ of the vinyl group of ring A of BV chromophore, and the sulfhydryl group of Cys82 is added at $C3^1$ of the vinyl group of ring A of BV. The double addition of the two sulfhydryls at the vinyl double bond causes loss of conjugation for ring A of BV with rings B, C, and D of BV, i.e. the conjugation length is shorter than normally singly bound BV. This situation makes BDFPs fluoresce in the FR region. BDFP1.6 fluoresces maximally around 665 nm and is much brighter than other BDFP1.1, 1.4, and 1.5, possibly owing to the double addition. Interestingly, the maximal fluorescence of BDFP1.6 can be shifted to ~705 nm after the cysteine residue at position 125 is mutated to a glycine, and the resulting variant, BDFP1.6(C125G), still keeps the high brightness as BDFP1.6. After further evolution of BDFP1.6(C125G), a new NIR FP is obtained and termed BDFP1.8 with a brightness much stronger than NIR BDFP1.1, 1.4, and 1.5. In mammalian cells this variant is even 2.4-fold brighter than the currently reported brightest NIR FP such as iRFP720.

> IL285. Invited Lecture

Symposium SENS-7 The Phytochrome Diversity: Mechanisms Light Signal Integration and Applications in Optogenetics (Wolfgang Gärtner)

SOLID-STATE NMR ON RED/FAR-RED-ABSORBING BILIN-BINDING GAF-DOMAIN PHOTOSENSORS

Authors: Chen Song[1], Qianzhao Xu[1,2], Kai-Hong Zhao[2], Jon Hughes[3], Jörg Matysik[1], Wolfgang Gärtner[1]
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Both phytochromes (Phys) and cyanobacteriochromes (CBCRs) are bilin-binding GAF- domain photosensors. Canonical Phys such as cyanobacterial and plant phytochromes exhibit red/far-red photocycles and the conserved PAS−GAF−PHY sensory module contains a unique knotted architecture (1−3). CBCRs, distantly-related to Phys, contain only the GAF domain but exhibit astonishing spectral diversity spanning almost the entire visible spectrum and near UV (4−6).

A large CBCR subgroup is formed as a red-absorbing dark state and photoconverted into a photoproduct absorbing green light. The gene 2699 from the cyanobacterium *Nostoc sp*. PCC7120 is the only one CBCR domain has been described to red shift the photoproduct absorbance from its red-absorbing dark state (7). The full-length protein contains three GAF domains and GAF1 and GAF3 alone can bind a PCB chromophore with absorption maxima in their dark states at ~650 nm. The 2699GAF1(2699g1) protein which undergoes an analogous Pr/Pfr-like photochromicity might serve as an ideal paradigm for the canonical Phys.

Here we present a comprehensive solid-state NMR study of the 2699g1 protein *in vitro* assembled with *u*-^{13}C,^{15}N-labeled PCB chromophore. On the basis of the complete ^{1}H, ^{13}C, and ^{15}N chemical shifts of the chromophore, we compare its electronic structure and the interactions with the binding pocket to a construct composed of both GAF1 and GAF2 (2699g1-2). The 2699g1-2 construct was generated in order to examine whether a potential interaction between the two GAF domains may alter the chromophore conformation. As a reference, we further compare the NMR data to those from the canonical Cph1 phytochrome that here serves as a reference protein.

Comparison of the available data of PCB chromophore in 2699g1, 2699g1-2 and Cph1 proteins in their respective red-absorbing states demonstrates that *1*) More pronounced structural heterogeneity of the chromophore in 2699g1, *2*) Possible coexistence of *S*- and *R*-stereoisomers at the ***A***-ring C3^{1} position of the 2699g1 bilin, whereas in 2699g1-2, the chiral center at C3^{1} is exclusively in the *R*-configuration, analogous to that of Cph1 (1); and *3*) The GAF2 domain mimics the overall organization of the PHY domain of the canonical Phys in protruding a tongue extension that partially seals the chromophore-binding pocket of GAF1. More results and future opportunities on the structural and functional interpretation of the available data of the 2699 proteins will be presented.

Conflicts of Interest

The authors declare no potential conflict of interest.

References

1. Essen LO, Mailliet J, Hughes J. (2008) *Proc Natl Acad Sci USA* **105**, 14709.
2. Burgie ES, Bussell AN, Walker JM, Dubiel K, Vierstra RD. (2014) *Proc Natl Acad Sci USA* **111**, 10179.
3. Rockwell NC, Lagarias JC (2010) *ChemPhysChem* **11**, 1172.
4. Ikeuchi M, Ishizuka T (2008) *Photochem Photobiol Sci* **7**, 1159.
5. Rockwell NC, Martin SS, Feoktistova K, Lagarias JC (2011) *Proc Natl Acad Sci USA* **108**, 11854.
6. Rockwell NC, Martin SS, Lagarias JC (2016) *Biochemistry* **55**, 3907.
7. Chen Y, et al. (2012) *FEBS J* **279**, 40.

> IL284. Invited Lecture

Symposium SENS-7 The Phytochrome Diversity: Mechanisms Light Signal Integration and Applications in Optogenetics (Wolfgang Gärtner)

STRUCTURAL CHANGES OBSERVED BY INFRARED DIFFERENCE SPECTROSCOPY OF A PHYTOCHROME-RELATED PROTEIN COMPOSED OF THREE GAF DOMAINS

Authors: Qianzhao Xu[1,3], Lucas Gött-Zink[2], Kai-Hong Zhao[3], Wolfgang Gaertner[1], Tilman Kottke[2]
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Cyanobacteriochromes (CBCRs) are members of the phytochrome protein family, found so far exclusively in cyanobacteria. They function as sensory photoreceptors. All2699 from *Nostoc* sp. PCC 7120 is a CBCR composed of three GAF domains, GAF1, GAF2, GAF3, out of which GAF1 and GAF3 each bind covalently a bilin (PCB) chromophore. The GAF1 domain undergoes a light-driven Pr to Pfr conversion just alike canonical phytochromes. We expressed different parts of this CBCR, i.e., GAF1 alone, GAF1-2 (a construct carrying fused GAF1 and GAF2), and All2699 (comprising all three GAF domains, GAF1, GAF2, and GAF3) in *E. coli* together with genes for phycocyanobilin biosynthesis. The spectroscopic properties and the light-induced conformational changes of the three different All2699 GAF constructs, furnished with PCB, were determined by UV-VIS absorption and by FT-infrared difference spectroscopy. GAF1-PCB and All2699-PCB have similar UV-vis absorption in both P_r (λ_{max} = 638 nm) and P_{fr} (λ_{max} = 685 nm) state. GAF1-2-PCB shows the same absorbance for the P_r state as GAF1-PCB and the full-length All2699-PCB, however, its P_{fr} absorption maximum is red-shifted by ca. 20 nm (λ_{max} = 705 nm) compared to all other constructs. Here we present the crucial structural changes of the protein after light activation of the chromphore detected by infrared difference spectroscopy. GAF1-PCB and All2699-PCB show very similar changes in their infrared difference spectra after light illuminating which means the structure of the two in both proteins chromophores change in a similar way. GAF1-2-PCB, instead, showed a very strong signal upon light activation, identified as a structural change of a formerly present β-sheet element being converted into an α-helical component. We propose that an extension of GAF2 in the GAF1-2 construct acts like the tongue in a phy domain in canonical phytochromes that is folded as β-sheet and is converted into an α-helix after red light illumination. As the full-length protein (All2699 with GAF1-3) presents the same UV-vis absorption maxima and similar infrared difference spectra as GAF1 alone, it implies that GAF3 can block the function of GAF2.

Conflicts of Interest

There are no conflicts of interests.

References

[1] Y. Chen *et al* (2012) FEBS J. 279, 40-54
[2] K. Anders *et al* (2013) J. BIOL. CHEM., 288, 35714–35725

> OC111. Oral Communication

Symposium SENS-7 The Phytochrome Diversity: Mechanisms Light Signal Integration and Applications in Optogenetics (Wolfgang Gärtner)

DEVELOPMENT OF OPTOGENETIC TOOLS FOR PLANT SYNTHETIC BIOLOGY

Authors: Rocío Ochoa-Fernández[1], Matias Zurbriggen[1]
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Plant synthetic biology is a nascent research area and therefore the development and implementation of engineering methods and synthetic tools still lags behind. In particular, optogenetic switches allow a precise quantitative regulation of cellular processes, such as gene expression, at high spatio-temporal resolution overcoming limitations of classical chemically inducible systems. While being widely applied in animal systems their implementation in plants imposes a challenge.

We have developed a synthetic light-inducible system for the targeted control of gene expression in plants based on the plant photoreceptor Phytochrome B and one of its interacting factors (PIF6), which is in the ON state upon illumination with red light (660 nm) and can be returned to the OFF state in white light. Additionally, we have expanded the toolbox of optogenetic switches by applying to plant cells a green light genetic switch, based on the CarH photoreceptor, that is in the ON state in dark and in the OFF state in presence of green light (525 nm) and the chromophore cobalamin. We present here the development of these first optogenetic systems for plants, and stress on the novel perspectives they present for the study of plant signaling processes, such as analysis of complex regulatory systems and metabolic pathways, with minimized invasiveness and high spatiotemporal resolution.

References

Chatelle, C. *et al.* A Green-Light-Responsive System for the Control of Transgene Expression in Mammalian and Plant Cells. *ACS Synth. Biol.***7**, 1349–1358 (2018).

Müller, K. *et al.* A red light-controlled synthetic gene expression switch for plant systems. *Mol Biosyst* **10**, 1679–88 (2014). Ochoa-Fernandez, R. *et al.* Optogenetics: Methods and Protocols. (ed. Kianianmomeni, A.) 125–139 (Springer New York, 2016).

> OC110. Oral Communication

Symposium SENS-7 The Phytochrome Diversity: Mechanisms Light Signal Integration and Applications in Optogenetics (Wolfgang Gärtner)

OPTOGENETICALLY REGULATED RECEPTOR TYROSINE KINASES, BASED ON BACTERIOPHYTOCHROME OF DEINOCOCCUS RADIODURANCE

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Introduction

Optically controlled mammalian receptor tyrosine kinases (opto-RTKs) allow non-invasive and reversible control of RTK activity with light (Leopold et al., 2018). To develop red-light controllable opto-RTKs we connected the photosensory core module (PCM) of the bacteriophytochrome of *Deinococcus radiodurance* (DrBphP) with the cytoplasmic domains of several RTKs. Upon illumination with red light (660 nm) the PCM of DrBphP undergoes structural changes, which result in the splaying apart of the PHY domains of the PCM core (Takala et al., 2014). These structural changes can be coupled to the enzymatic activity of the RTK cytoplasmic domains, attached to the C-termini of PHY domains of DrBphP-PCM. We have connected cytoplasmic domains of the NGF receptor TrkA, epidermal growth factor receptor (EGFR) and insulin receptor (IR) with the DrBphP PCM with (EAAAK)4 helical linkers and proved the ability of resultant opto-RTKs termed Dr-RTKs to regulate ERK cascade in the light-dependent manner.

Methods

For the evaluation of ERK activity mammalian cells were transfected with plasmids encoding Dr-RTKs and reporter plasmids pFR-Luc and pFA-Elk-1 as described (Leopold et al., 2019). Light-dependent activation of PI3K and Ca^{2+} signaling by Dr-RTKs in cell culture and light activation and imaging of ERK pathway in freely moving mice is described elsewhere (Leopold et al. 2019).

Results and discussion

Dr-RTKs activate ERK cascade upon NIR illumination and downregulate it upon red light illumination. Dr-RTKs fast and reversibly control Ca^{2+} level in HeLa cells and activation of PI3K/Akt pathway. 1 min of NIR illumination was enough to induce ERK activation in HeLa cells transfected with Dr-RTKs. Finally, DrBphP-based RTKs are able to control ERK cascade in freely moving mice in the light-dependent manner, with 660 nm light switching ERK cascade OFF and with NIR light switching ERK cascade ON.

Conclusion

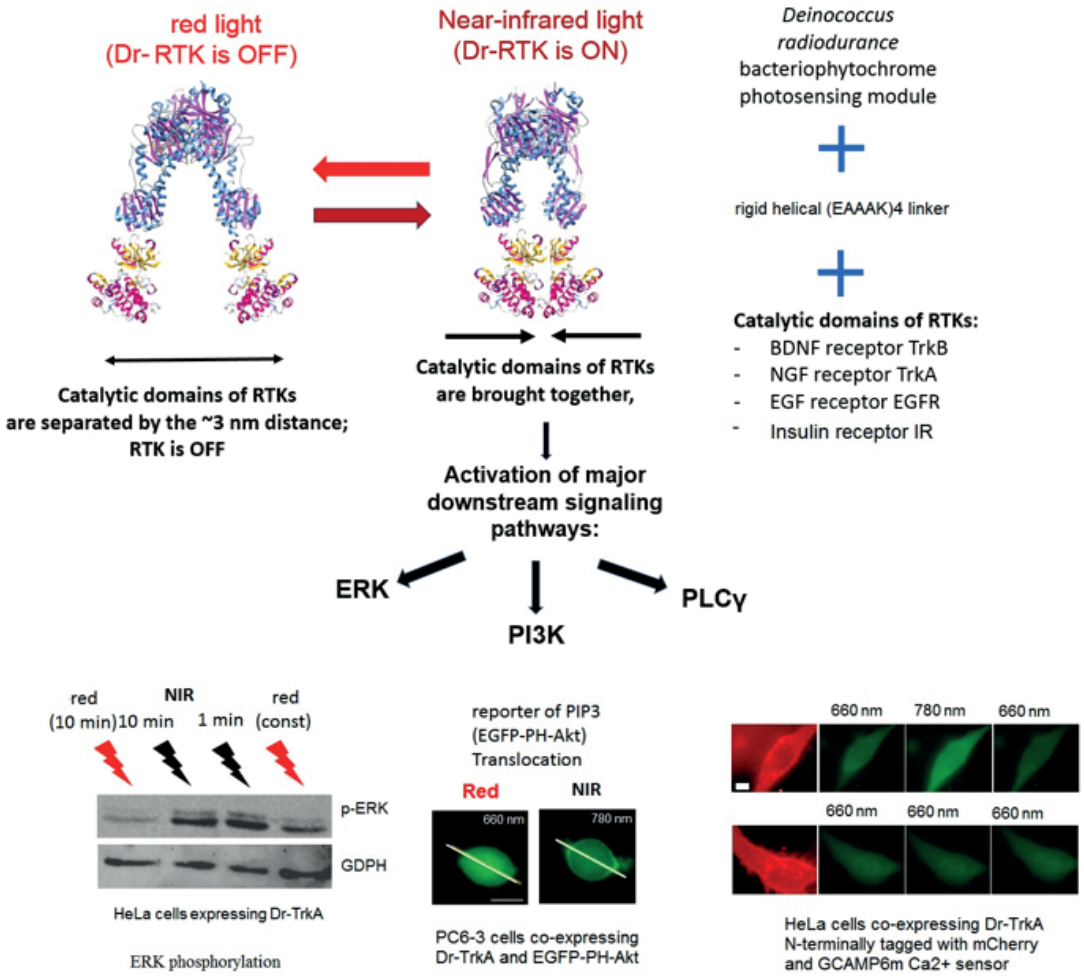
Dr-RTKs use artificial rigid helical linkers to control activity of the attached cytoplasmic RTK domains. Illumination with red light causes separation of RTK domains and their inactivation, while illumination with NIR light brings them together and leads to their activation and to activation of the canonical downstream RTK signaling.

Conflict of interest

The authors declare no conflict of interest

Continued

References
Leopold, A.V., Chernov, K.G., Shemetov, A.A., and Verkhusha, V.V. (2019). Neurotrophin receptor tyrosine kinases regulated with near-infrared light. *Nat Commun* 10(1), 1129. doi: 10.1038/s41467-019-08988-3.
Leopold, A.V., Chernov, K.G., and Verkhusha, V.V. (2018). Optogenetically controlled protein kinases for regulation of cellular signaling. *Chem Soc Rev* 47(7), 2454-2484. doi: 10.1039/c7cs00404d.
Takala, H., Lehtivuori, H., Hammaren, H., Hytonen, V.P., and Ihalainen, J.A. (2014). Connection between absorption properties and conformational changes in Deinococcus radiodurans phytochrome. *Biochemistry* 53(45), 7076-7085. doi: 10.1021/bi501180s.



> P136. Poster

Symposium SENS-7 The Phytochrome Diversity: Mechanisms Light Signal Integration and Applications in Optogenetics (Wolfgang Gärtner)

BIOPHYSICAL STUDIES ON BACTERIAL BILIVERDIN-BINDING PHYTOCHROMES

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Bacterial photoreceptors binding open-chain tetrapyrroles (bilins) as chromophores are related to plant phytochromes (phy) as they are photochromic and their primary photochemistry consists of a *Z/E* isomerisation around the bilin 15=16 double bond. The chromophore is embedded in all cases within a so-called GAF domain with a typical α/β fold. Different to the canonical plant phys which invariably bind phytochromobilin and switch between a red (R) and a far red (FR) absorbing form, the bacterial bilin-binding photoreceptors exhibit a much wider variety of spectroscopic and functional properties, and bind diverse bilin chromophores, e.g., phycocyanobilin (PCB) and biliverdin (BV). In particular, BV-binding photoreceptors present the most red-shifted spectrum, reaching the near infra-red (NIR) range in the photoactive form. This makes these phytochromes very well suited for biomedical applications (1). Here we report steady-state and time-resolved spectroscopic measurements on selected bacterial BV-binding photoreceptors, representatives for four variations of this photoreceptor family: **a.** a phy and a bathy-phy from *Pseudomonas* strains with R/FR photochromism; **b**. a "bacterio" phytochrome from the fungus *Aspergillus nidulans,* a eukaryotic organism with photochemistry akin to the *Pseudomonas syringae* protein; **c.** a novel phy from *Methylobacterium radiotolerans* with FR/NIR photochromism. In particular, nanosecond time-resolved absorption spectroscopy has revealed kinetics and spectral features of transient species after photoactivation for both the directions of conversion: the conversions of all these BV-phytochromes in the time range 1 µs – 400 ms seem to be more simple than those from plant phytochromes (*oat* phyA) or from cyanobacteria (Cph1, CphA) (2)(3), in some cases travelling through only one observable intermediate in the R to FR conversion.

References

(1) Chernov, K.G. et al. (2017) Chem. Rev. 117 6423-6446.
(2) Gärtner, W. and Braslavsky, S.E. (2003) In: Photoreceptors and light signalling, Batschauer, A. (ed.). Compr. Series Photochem. Photobiol. Sci., Vol. 3, Batschauer, A. (ed.), Häder, D.-P. and Jori, G. (series eds.), Royal Soc. Chemistry, Cambridge, UK, pp. 136-180.
(3) Remberg, A. et al. (1997) Biochemistry 36 13389-13395.

> P137. Poster

Symposium SENS-7 The Phytochrome Diversity: Mechanisms Light Signal Integration and Applications in Optogenetics (Wolfgang Gärtner)

LIGHT-DEPENDENT REGULATION OF FLOCCULATION IN SYNECHOCYSTIS SP. PCC6803 AND THE IMPORTANCE FOR THE NUTRIENT STATE OF THE CELLS.

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Presenting Author: Fabian D. Conradi
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Bacterial aggregate formation has been a topic of considerable interest recently and may well represent the cusp of multicellularity. Here, we explore the formation of flocs, floating bacterial aggregates, in the unicellular model cyanobacterium *Synechocystis sp.* PCC6803 by developing an assay to measure flocculation. We show that light colour is an important regulatory factor in floc formation, up-regulating flocculation in blue light relative to green light. The blue-green photoconvertible cyanobacteriochrome Cph2 is shown to be largely responsible for this effect, likely due to its role in regulating levels of the secondary messenger cyclic di-GMP. We further show that *Synechocystis* cells in the centre of flocs tend to experience stress and provide evidence this can be caused by nutrient stress. The wavelength-based regulation of light is likely an important factor in regulating floc size via self-shading to avoid the damaging impact of excessive blue light but poses interesting questions regarding nutrient distribution in such a scenario.

> P138. Poster

Symposium SENS-7 The Phytochrome Diversity: Mechanisms Light Signal Integration and Applications in Optogenetics (Wolfgang Gärtner)

PSEUDOMONAS SYRINGAE PV. TOMATO BACTERIOPHYTOCHROMES DOWNREGULATE BACTERIAL MOTILITY AND INFECTIVITY DURING PLANT-PATHOGEN INTERACTION

Authors: Daniela Ceresini[1], Ada Ricci[1], Lucia Dramis[1], Francesca Degola[1], Aba Losi[1]
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The foliar hemibiotrophic pathogen *Pseudomonas syringae* pv. tomato DC3000 (*Pst*) leads to consistent losses in tomato crops and this urges to multiply the researches on the physiological bases of its infectiveness. It has been already demonstrated that light perception plays a crucial role in many physiological processes, even in non-phototrophic organisms. *Pst* is equipped with red/far-red (R/FR) light sensing bacteriophytochromes (BphP), binding biliverdin as chromophore and mimicking the photosensing ability of host plants. Here we report the study of the effect of different light conditions on the swarming motility of mutant strains of *P. syringae* lacking of the photosensory Bphy1 or Bphy2 or both proteins or heme-oxygenase-1 (HO) catalysing formation of bilins, respectively, as regards to the wild type (WT). Each of the mutants shows stronger virulence than *Pst*WT evidenced by the macroscopic damages caused in the infected leaves of tomato plants. Moreover, they rapidly move inside the infected plants, as necrotic spots in host tissues distant from the infection site appear faster than that due to the WT infection. These results indicate that bacteriophytochromes downregulate bacterial infectivity and invasiveness within the infected leaves and underscore the importance of *Pst* photoreceptors in responding to environmental light inputs.

> P139. Poster

Symposium SENS-7 The Phytochrome Diversity: Mechanisms Light Signal Integration and Applications in Optogenetics (Wolfgang Gärtner)

ELUCIDATING CYANOBACTERIAL-PHYTOCHROME CPH1-MEDIATED INTRACELLULAR SIGNALING PATHWAYS IN SYNECHOCYSTIS SP. PCC 6803

Authors: Nibedita Priyadarshini[1,2], Thomas Wallner[1], Annegret Wilde[1]
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Phytochromes are red, far-red photoreceptors that act as light sensitive switches to regulate diverse cellular mechanisms. Earlier they were believed to be present only in plants however, whole genome sequencing analysis of the cyanobacterium *Synechocystis* sp. PCC 6803 revealed the presence of a first prokaryotic phytochrome gene sequence called cyanobacterial phytochrome 1 (*cph1)*. The gene product, Cph1, has an N-terminal sensory module regulated by light and a C-terminal histidine kinase module, which is typical for bacterial two-component sensory kinases. Since the discovery of Cph1 over almost two decades ago, not much is known about its interacting partners nor about the cellular pathways it regulates.

Cph1 has its own cognate response regulator, Rcp1 that has been speculated over the years, to be involved in downstream regulation. While most response regulators possess a DNA-binding domain mediating transcriptional regulation, Rcp1 lacks any known signal output domain. This suggests that downstream regulation by Rcp1 might involve protein-protein interactions. We have expressed FLAG- and eYFP-tagged Cph1 and Rcp1 proteins in *Synechocystis* sp. PCC 6803 respectively and identified putative interacting proteins by mass spectrometry. Potential interaction partners include, amongst others, enzymes of the glycogen metabolism and components of the circadian clock oscillator. On the other hand, expression of the *cph1-rcp1* operon is upregulated in the dark and is itself controlled by the circadian output regulator RpaA. Therefore, we will discuss our data in line with potential involvement of Cph1-induced signal transduction pathway in adaptation to dark growth conditions.

> IL291. Invited Lecture

Symposium SENS-8 Cyanobacteriochromes. Characterization, Chromatic Adaptation and Applications in Optogenetics (J. Clark Lagarias)

RATIONAL COLOR-TUNING DESIGNS ON TWO CYANOBACTERIOCHROME LINEAGES BASED ON THEIR NATURAL DIVERSITY

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Introduction

Linear tetrapyrrole-binding cyanobacteriochrome (CBCR) photoreceptors cover the entire UV-to-visible spectrum[1]. Only the GAF domains are enough for chromophore incorporation, and their sequences are categorized into several lineages. Notably, spectral diversities even within each lineage have been identified. In this talk, we focus on expanded red/green (XRG) and DpxA lineages. Although typical XRG molecules bind phycocyanobilin (PCB) and show red/green reversible photoconversion, atypical far-red/orange reversible photoconversion has been identified, which is established by biliverdin (BV) incorporation instead of PCB[2]. On the other hand, typical molecules within the DpxA lineage possess a second Cys residue important for isomerization of PCB to phycoviolobilin (PVB) and reversible Cys adduct formation[3]. In this lineage, blue/teal and green/teal photoconversions have been identified. Although these molecules bind PVB in both cases, blue/teal and green/teal photoconversions proceed with and without reversible Cys adduct formation, respectively.

Methods

We characterized the wild-type and mutant molecules of the various GAF domains purified from the BV- and PCB-producing *E. coli*.

Results and Discussion

First, we focused on the BV-binding molecules within the XRG lineage. Based on the structural and sequence information, comprehensive mutagenesis study revealed that replacement of only four residues was enough for conversion from BV-rejective molecules into BV-acceptable molecules. The crystal structure of one of such engineered molecules identified unusual covalent bond linkage, which resulted in deep BV insertion into the protein pocket. The four mutated residues contributed reducing steric hindrances derived from the deeper insertion. We introduced these residues into some molecules, and one of them produced bright near-infrared fluorescence.

Second, we focused on the DpxA lineage. We found that AM1_1499g1 did not possess the second Cys residue in spite of belonging to this lineage. AM1_1499g1 covalently bound PCB and showed orange/green reversible photoconversion, indicating no isomerization activity nor reversible Cys adduct formation. Site-directed mutagenesis of AM1_1499g1 succeeded in designing sextuple photoconvertible molecules, orange/green, yellow/teal, blue/teal, orange/yellow, yellow/green, and blue/green photoconversions that were modified with regard to binding chromophore species, reversible Cys adduct formation and ring D twisting.

Conclusions

We have succeeded in rational color-tuning designs on the two lineages. This study would provide not only basic insights into the photoconversion mechanism but also promising strategy to develop optogenetic and bio-imaging tools.

References

1. Fushimi & Narikawa (2019) *Curr. Opin. Struct. Biol.*
2. Narikawa et al. (2015) *Sci. Rep.*, Fushimi et al. (2016) *Front. Microbiol.*
3. Rockwell et al. (2012) *Biochemistry*, Wiltbank & Kehoe (2016) *mBio*, Hasegawa et al. (2018) *J. Biol. Chem.*

> IL296. Invited Lecture

Symposium SENS-8 Cyanobacteriochromes. Characterization, Chromatic Adaptation and Applications in Optogenetics (J. Clark Lagarias)

THE ORIGIN OF THE RED/GREEN SPECTRAL TUNING IN THE CYANOBACTERIOCHROME SLR1393G3

Authors: Igor Schapiro[1], Christian Wiebeler[1], Aditya Gopalkrishna Rao[1]
Presenting Author: Igor Schapiro
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Phytochromes are a widespread family of responsive photoreceptors initially discovered in plants.[1] Canonical phytochromes utilize covalently attached bilin chromophores that undergo a reversible photoconversion between red- (Pr) and far-red-absorbing (Pfr) forms. Recently, a new subgroup of the phytochrome superfamily called cyanobacteriochromes (CBCRs) was found in cyanobacteria.[2-3] Despite the phylogenetic relation to canonical phytochromes, the CBCR family stands out because of the compact protein size, which is restricted to a single GAF domain, that is contrast to the PAS-GAF-PHY architecture of canonical phytochromes. Another characteristic is that CBCRs exhibit an unprecedented diversity in the spectral tuning, which spans the entire visible spectrum and extends from the near-IR to the near-UV region.[2,4-6] One frequently present subfamily of CBCRs exhibits a red-absorbing dark state (Pr) and a green-absorbing photoproduct state (Pg), showing a shift in absorption of more than 100 nm.[7] Thus the photoproduct absorption undergoes a hypsochromic shift instead of the bathochromic one observed for canonical phytochromes. This led to the question as to which factors trigger such a reverse shift.

To understand the origin of the spectral shift we used the hybrid quantum mechanics/molecular mechanics simulations.[8] Our calculations revealed that the effective conjugation length in the chromophore becomes shorter upon conversion from the red to the green form. This is related to the planarity of the entire chromophore. A large distortion was found for the terminal pyrrole rings A and D; however, the D ring contributes more strongly to the photoproduct tuning, despite a larger change in the twist of the A ring. Our findings implicate that the D ring twist can be exploited to regulate the absorption of the photoproduct. Hence, mutations that affect the D ring twist can lead to rational tuning of the photoproduct absorption, allowing the tailoring of cyanobacteriochromes for biotechnological applications such as optogenetics and bioimaging.

References

1. Rockwell, N. C., Su, Y.-S., Lagarias, J. C., *Annu. Rev. Plant Biol.* 2006, 57, 837– 858.
2. Ikeuchi, M., Ishizuka, T., *Photochem. Photobiol. Sci.* 2008, 7, 1159– 1167.
3. Rockwell, N. C., Ohlendorf, R., Möglich, A., *Proc. Natl. Acad. Sci. USA* 2013, 110, 806– 807.
4. Rockwell, N. C., Martin, S. S., Feoktistova, K., Lagarias, J. C., *Proc. Natl. Acad. Sci. USA* 2011, 108,11854– 11859.
5. Enomoto, G., Hirose, Y., Narikawa, R., Ikeuchi, M., *Biochemistry* 2012, 51, 3050– 3058.
6. Rockwell, N. C., Martin, S. S., Gulevich, A. G., Lagarias, J. C., *Biochemistry* 2012, 51, 1449– 1463.
7. Rockwell, N. C., Martin, S. S., Lagarias, J. C., *Biochemistry* 2012, 51, 9667– 9677.
8. Wiebeler, C., Gopalkrishna Rao, A., Schapiro, I., Angew. Chem. Int. Ed. 2019, 58, 1934–1938.

> IL295. Invited Lecture

Symposium SENS-8 Cyanobacteriochromes. Characterization, Chromatic Adaptation and Applications in Optogenetics (J. Clark Lagarias)

UNUSUAL CHROMOPHORE CONFIGURATION AND PHOTOCONVERSION MECHANISM IN FAR RED CYANOBACTERIOCHROME

Authors: Xiaojing Yang[1], Sepalika Bandara[1], Xiaoli Zeng[1], Nathan Rockwell[2], Zhong Ren[1], Heewhan Shin[1], Clark Lagarias[2]
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1) Univesity of Illinois at Chicago 2) University of California Davis

Cyanobacteriochromes (CBCRs) are small photoswitchable bilin-based sensors that regulate diverse biological processes in cyanobacteria. Owing to deep tissue penetration of Far-Red (FR) light, newly identified FR-absorbing CBCRs are highly desirable protein scaffolds for design of optical agents for biomedical imaging and optogenetic applications. Presently, nothing is known how FR perception by CBCRs is achieved at the molecular level. We have determined the crystal structures of the FR CBCR Anacy2551g3 from *Anabaena cylindrica* PCC 7122 determined in two distinct light signaling states. I will discuss the unusual chormophore conformation that accounts for the extremely far-red absorption as well as the photoconversion mechanism unique to this novel family of far-red cyanobacteriochromes.

> IL297. Invited Lecture

Symposium SENS-8 Cyanobacteriochromes. Characterization, Chromatic Adaptation and Applications in Optogenetics (J. Clark Lagarias)

PLANARIZATION IS AN ALTERNATIVE PHOTOCYCLE INITIATION MECHANISM IN THE DYNAMICS OF THE TRI-CYSTEINE VIOLET/BLUE PHOTOSWITCHING CYANOBACTERIOCHROME FROM MOOREA PRODUCENS

Authors: Delmar Larsen[1], J. Clark Lagarias[1], Nathan Rockwell[1], Julia Kirpich[1]
Presenting Author:Delmar Larsen
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Photoreceptor proteins are ideal systems to study functional reaction dynamics since they can be triggered with short pulses of light, and their signaling activity can be directly followed via spectroscopic techniques[JCL1] . This presentation will discuss the femtosecond and cryokinetics photodynamics of the 6th GAF domain of LYNGBM3L_56870 from *Moorea producens* of the strain 3L LYNGBM3L_56870g6 (moorea_g6; UniProtKB F4XZC1) which is a representative of a \'tri-cysteine\' cyanobacteriochrome (CBCR) subfamily. CBCRs in this family have both a conserved thioether linkage with the phycocyanobilin (PCB) chromophore precursor using the 1st cysteine and a second linkage to C10 carbon of PCB using the \'insert-cysteine\' and \'DXCF-cysteine\' (Asp-Xaa-Cys-Phe) motif in dark-adapted ($^{15Z}P_v^{ins\text{-}cys}$) and light-adapted ($^{15E}P_b^{DXCF}$) states, respectively. Moorea_g6\'s femtosecond dynamics in the forward ($^{15Z}P_v^{ins\text{-}cys} \rightarrow$) direction exhibit significant spectral (~100 nm difference) and kinetic heterogeneity. We have evaluated five hypotheses for the origin of this heterogeneity by exploiting high-level quantum calculations. One population can be assigned to the rapid dissociation of the cysteine-adduct at C10 and subsequent planarization of the chromophore. This is a novel isomerization dynamics mechanism not previously seen for CBCRs. While both planarization and isomerization mechanisms generate independent primary photointermediates for moorea_g6, the two pathways merge into a single pathway in subsequent dynamics to mutually generate the terminal $^{15E}P_b^{DXCF}$ photostate. Interestingly, similar dynamics are observed in other CBCRs including the NpF2164g3 (UniProtKB B2J668) from *Nostoc punctiforme*, suggesting that this mechanism may occur more widely in dually linked CBCRs.

> IL292. Invited Lecture

Symposium SENS-8 Cyanobacteriochromes. Characterization, Chromatic Adaptation and Applications in Optogenetics (J. Clark Lagarias)

DIVERSE CHROMATIC ACCLIMATION PROCESSES RESPONDING TO GREEN AND RED LIGHT IN THE CYANOBACTERIA PHYLUM

Authors: Yuu Hirose[1], Song Chihong[2], Mai Watanabe[3], Chinatsu Yonekawa[4], Kazuyoshi Murata[2], Masahiko Ikeuchi[5], Toshihiko Eki[1]
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1) Toyohashi University of Technology 2) National Institute for Physiological Sciences 3) University of Freiburg 4) Keio University 5) The University of Tokyo

Introduction

Cyanobacteria have evolved various photoacclimation processes to perform oxygenic photosynthesis under different light environments. Chromatic acclimation (CA) is widely recognized and ecologically important type of photoacclimation, where cyanobacteria alter the absorbing light colors of their supermolecular antenna complex called phycobilisome. In the 1970s, cyanobacteria strains containing both phycoerythrin (PE) and phycocyanin (PC) were classified based on their responses to green and red light: CA1 regulating neither PE nor PC, CA2 regulating PE but not PC, and CA3 regulating both PC and PE [1]. However, diverse CA variants other than CA2 and CA3 have been characterized to date. Marine *Synechococcus* and *Prochlorococcus* alter the chromophorylation of PE in response to blue and green light, which is designated as CA4 [2,3]. *Acaryochloris* increases the PC content under orange-red light [4], which can be classified CA5. Certain cyanobacteria produce the far-red-absorbing types of phycobilisome and also photosystems containing chlorophyll *f* [5], which is designated as far-red light photoacclimation (FaRLiP) and can be classified as CA6. Our research motivation is how diverse are the molecular processes of CA in the cyanobacteria phylum.

Results and Discussion

We surveyed the gene composition of the *ccaS*/*ccaR* photosensing gene cluster for CA2 in ~1300 strains of cyanobacteria genomes [6, 7]. We identified a unique *ccaS*/*ccaR* cluster encoding yellow-green-absorbing phycoerythrocyanin (PEC) and a rod-membrane linker protein (CpcL) for the rod-shaped form of phycobilisome [8]. Using the cyanobacterium *Leptolyngbya* sp. PCC 6406, we revealed novel CA variants regulating PEC (CA7) and the rod-shaped phycobilisome (CA0), which maximize yellow-green light-harvesting capacity and balance the excitation of photosystems, respectively [9]. The distributions of CA gene clusters in 445 cyanobacteria genomes revealed eight CA variants responding to green and red light, which are classified based on the presence of PEC, PE, *cpcL* and CA photosensor genes [9]. Phylogenetic analysis further suggested that the emergence of CA7 was a single event and preceded that of heterocystous strains, whereas the acquisition of CA0 occurred multiple times [9]. These results offer novel insights into the diversity and evolution of the complex cyanobacterial photoacclimation mechanisms

References

1. Tandeau de Marsac, N. (1977). J. Bacteriol. 130:82-91.
2. Everroad, C. et al. (2006). J. Bacteriol. 188:3345-3356.
3. Shukla, A. et al. (2012). Proc. Natl. Acad. Sci. U. S. A. 109:20136-20141.
4. Duxbury, Z. et al. (2009). Photosynth. Res. 101:69-75.
5. Gan, F. et al (2014). Science 345:1312-1317.
6. Hirose, Y. et al. (2008). Proc. Natl. Acad. Sci. U. S. A. 105:9528-9533.
7. Hirose, Y. et al. (2010). Proc. Natl. Acad. Sci. U. S. A. 107:8854-8859.
8. Watanabe, M. et al. (2014). Proc. Natl. Acad. Sci. U. S. A. 111:2512-2517.
9. Hirose, et al. (2019) Mol. Plant. in press.

> IL293. Invited Lecture

Symposium SENS-8 Cyanobacteriochromes. Characterization, Chromatic Adaptation and Applications in Optogenetics (J. Clark Lagarias)

THE ROLE OF CYANOBACTERIOCHROMES IN PHOTOTAXIS

Authors: Annegret Wilde[1], Annik Jacob[1], Nils Schuergers[1], Thomas Wallner[1], Veronika Angerer[1], Conrad Mullineaux[2]
Presenting Author: Annegret Wilde
1) Institute of Biology III, University of Freiburg 2) Queen Mary University of London

Cyanobacteria can move over surfaces in order to find optimal conditions for photosynthesis. This light-dependent twitching motility relies on the dynamic polar assembly of type IV pili. Unlike anoxygenic phototrophs, cyanobacterial cells are able to accurately perceive the direction of a light source due to optical lensing effects and therefore they move directly and predictably to a light source. In cyanobacterial phototaxis, we hypothesize that light lensing controls localized assembly and disassembly of the pilus apparatus depending on the light direction. In the cyanobacterium *Synechocystis*6803, cyanobacteriochromes and a BLUF-domain photoreceptor control positive and negative phototaxis via chemotaxis-like regulators. We have identified the localization of these CheY-like regulators and their interaction with type IV pili via the motor ATPase PilB1.We present data which suggests that light and ethylene control the concentration of active CheY-like regulators. We discuss the formation of cell polarity and directed movement in the cyanobacterium *Synechocystis* 6803 by spatial differences in the amount of CheY homologs and their binding to the type IV pili. Thereby, lensing effects induce spatial activation of these phototaxis regulators.Further, we show that light-dependent c-di-GMP production by the cyanobacterial phytochrome Cph2 modulate motility of cyanobacterial cells and that the circadian clock might feed additional information into the system.

> IL294. Invited Lecture

Symposium SENS-8 Cyanobacteriochromes. Characterization, Chromatic Adaptation and Applications in Optogenetics (J. Clark Lagarias)

OPTOGENETIC APPLICATIONS OF CYANOBACTERIOCHROMES

Authors: Jeffrey Tabor[1]
Presenting Author: Jeffrey Tabor
1) Rice University

Photosensing bacteria utilize cyanobacteriochromes to detect light from the ultraviolet to near infrared and mount diverse physiological responses. Many such photoreceptors are associated with two-component signal transduction pathways, wherein the photoreceptor regulates the activity of a response regulator protein, which in turn regulates gene expression. We have utilized a variety of synthetic biology methods to port the UirSR and CcaSR systems from their native hosts into the model bacteria E. coli and B. subtilis. In each case, we utilize previously established methods for production of the phycocyanobilin chromophore. We have used these photoreceptor systems to achieve precise spatial and temporal control of gene expression. We are now applying these techniques to uncover novel signal processing features of the B. subtilis sporulation pathway, and to directly manipulate gut bacterial metabolism in live C. elegans worms.

> OC112. Oral Communication

Symposium SENS-8 Cyanobacteriochromes. Characterization, Chromatic Adaptation and Applications in Optogenetics (J. Clark Lagarias)

DIRECTIONAL LIGHT PERCEPTION FOR PHOTOTAXIS IN CYANOBACTERIA

Authors: Conrad Mullineaux[1], Helder Camen[1], Ruiqian Zhou[1], Arjen Bader[2], Alan Lowe[3], Annegret Wilde[4]
Presenting Author: Conrad Mullineaux
1) Queen Mary University of London, UK 2) University of Wageningen, the Netherlands 3) University College London, UK 4) University of Freiburg, Germany

Introduction

Many cyanobacteria use Type IV pili to move on surfaces, and are capable of phototaxis, using their twitching motility to move either towards or away from a light source. Phototaxis requires directional light perception, and we recently showed that individual cells of the spherical cyanobacterium *Synechocystis* sp. PCC6803 can accurately perceive the position of a light source due to micro-lensing: the cell focuses an image of the light source at the opposite periphery of the cell, where it is detected by photoreceptors in or close to the plasma membrane (1). How can *Synechocystis* act as such an effective micro-lens, and which are the directional photoreceptors?

Methods

Fluorescence Lifetime Imaging Microscopy (FLIM) and quantitative phase imaging to map the refractive index of *Synechocystis* cells; 3D-Finite Difference Time Domain (FDTD) simulations to model the lensing properties of *Synechocystis* cells; molecular genetics and phototaxis assays to identify the photoreceptors essential for directional light perception.

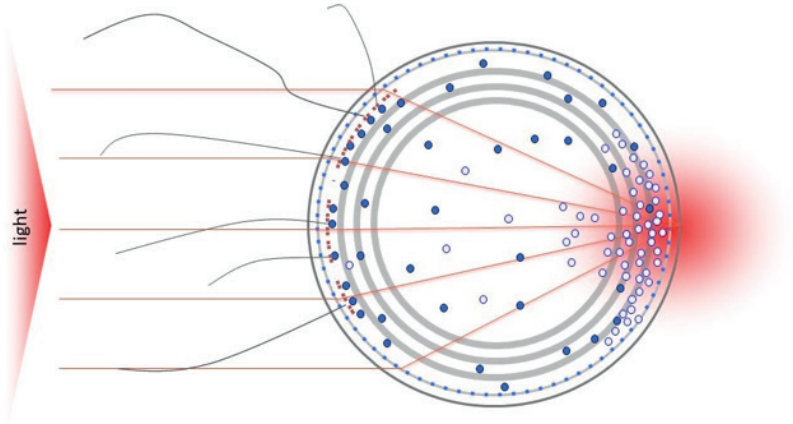
Results

The refractive index (*n*) of *Synechocystis* cells is not uniform. In the central cytoplasm, *n* ~1.4 (typical for the bacterial cytoplasm) while in the surrounding thylakoid membrane layers *n* is unusually high (~1.5), probably due to the very high concentration of lipid and protein in this region. FDTD simulations show that a model *Synechocystis* cell with these properties acts a very effective microlens, even when the cell is immersed in water. Mutagenesis experiments with multiple knockouts indicate that *Synechocystis* has multiple directional photoreceptors, with the cyanobacteriochrome PixJ and the BLUF protein PixD among the strongest candidates.

Conclusion

Directional light perception in *Synechocystis* is enabled by the specific optical properties of the cell, which allow it to act as a robust micro-lens. The “retina” of the cell is populated by at least 2 directional photoreceptors, which enable a form of colour vision in this bacterium.

References
1. Schuergers N et al. (2016) *eLife* 2016;5:e12620



> P140. Poster

Symposium SENS-8 Cyanobacteriochromes. Characterization, Chromatic Adaptation and Applications in Optogenetics (J. Clark Lagarias)

IN SEARCH OF THE DIRECTIONAL PHOTORECEPTOR(S) FOR NEGATIVE PHOTOTAXIS IN SYNECHOCYSTIS PCC6803

Authors: Ruiqian Zhou[1], Annegret Wilde[2], Conrad Mullineaux[1]
Presenting Author: Ruiqian Zhou
1) School of Biological & Chemical Sciences, Queen Mary, University of London, Mile End Rd, London, UK. 2) Institute for Biology III, Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany.

Background

In the cyanobacterium *Synechocystis* sp. PCC6803, positive and negative phototaxis are induced by different wavelengths and intensities of light. Different response regulators activate the Type IV pili (T4P) to initiate directional movement. Mutagenesis studies show that several photoreceptors are implicated in the control or tuning of phototaxis, but it is not clear which photoreceptors are responsible for directional light sensing. Such photoreceptors should be located around the cell periphery to sense the image of a light source which is focused at the cell surface (*eLife* 2016; 5:e12620). Mutants lacking each of the known photoreceptors retain directional movement in response to light, which indicates that no known photoreceptor is solely responsible for sensing light. To investigate the mechanism of sensing direction for negative phototaxis in *Synechocystis* sp. PCC6803, we focus on the ultraviolet photoreceptor UirS, the blue light photoreceptor PixD, and the blue/green (and possibly red) photoreceptor PixJ. Multiple knock-out mutants were constructed to explore potential synergistic effects. Localization of photoreceptors would also help to understand their role in light sensing.

Method

The knock-out mutants Δ*uirS*, Δ*pixD*, Δ*uirS*Δ*pixJ*, Δ*pixD*Δ*pixJ*, Δ*uirS*Δ*pixD*, Δ*uirS*Δ*pixD*Δ*pixJ* were constructed. Motility assays under white and UV-A (315-400 nm) light were performed. A UirS-eYFP fusion was expressed in the native locus. Δ*uirS* was complemented with uirS-eYFP expressed with a controllable promoter. Localization of UirS-eYFP was observed by confocal microscopy.

Results

Δ*uirS*Δ*pixJ* is still able to do negative phototaxis under UV-A and white light. UirS-eYFP is unevenly distributed on the cell membrane, and therefore could not be an efficient directional light sensor

Conclusion

UirS cannot be the sole directional light sensor for negative phototaxis, suggesting that a combination of PixD and UirS, or PixD and PixJ, or UirS, PixD and PixJ all together fulfill this function. Studies of the directional light-sensing abilities of Δ*pixD*Δ*pixJ*, Δ*uirS*Δ*pixD*, and Δ*uirS*Δ*pixD*Δ*pixJ* are underway.

References

Schuergers, N. et al. Cyanobacteria use micro-optics to sense light direction. eLife 5, 16, doi:10.7554/eLife.12620 (2016).

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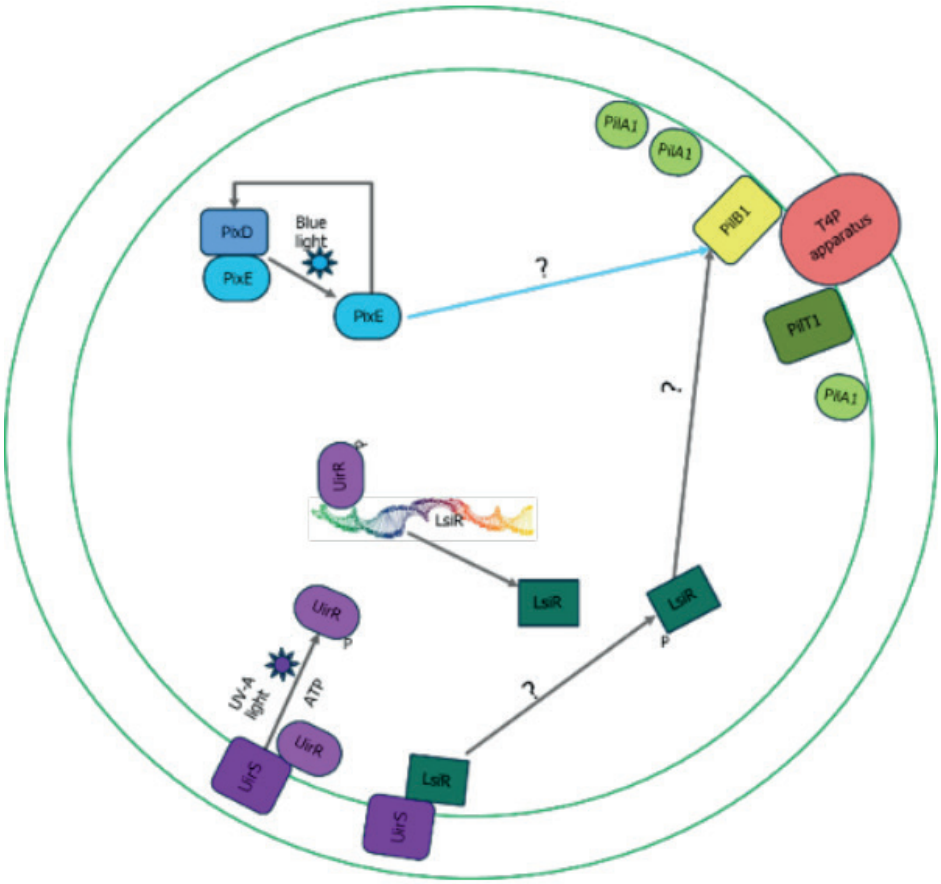
PilA1
PilA1
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T4P apparatus
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PixD
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> P141. Poster

Symposium SENS-8 Cyanobacteriochromes. Characterization, Chromatic Adaptation and Applications in Optogenetics (J. Clark Lagarias)

HYBRID QM/MM STUDY OF THE PHOTOCHEMISTRY IN THE CYANOBACTERIOCHROME ALL2699G1

Authors: Avishai Barnoy[1], Christian Wiebeler[1], Aditya G. Rao[1], Igor Schapiro[1]
Presenting Author: Avishai Barnoy
1) Institute of Chemistry, The Hebrew University of Jerusalem

Phytochromes (Phys) are photosensors found in plants, bacteria, and fungi, first discovered in plants. They possess a three-domain structure, with one of them covalently binding an open-chain tetrapyrrole as a chromophore for light absorption. Canonical Phys exhibit reversible photoconversion between red (P_r) and far-red absorbing (P_{fr}) forms[1]. Recently, a sub-group of Phys was discovered called cyanobateriochromes (CBCRs). CBCR requires the chromophore binding GAF domain for complete photochemistry. CBCRs can be classified in at least four categories based on the typical absorption of dark state and photoproduct: red/green, green/red, blue/orange (insert-Cys), and blue/green (DXCF). Recently, a new subfamily of CBCRs was found that switches from a red absorbing dark state (P_r) to a far-red absorbing photoproduct (P_{fr}), like Phys.[2] Thus, in the all2699g1 CBCR a complete red/far-red photocycle is achieved with just one instead of three domains.

In this contribution we have studied all2699g1 using hybrid quantum mechanics/molecular mechanics in combination with an *ab initio* wave function method to unravel the factors governing its unique photochemistry. Such an approach has already proven to be successful to obtain a molecular understanding of the photoproduct tuning in Slr1393g3.[3] Hence, we have performed sampling in the ground state to explore the conformational flexibility of all2699g1 and then compare the results obtained for Slr1393g3. Subsequently, we have computed UV/Vis and CD spectra to analyze how the different conformations can be analyzed spectroscopically.

References

(1) Heintzen, C. Plant and Fungal Photopigments. *WIREs Membr. Trans. Signal.* **2012**, *1* (4), 411–432.
(2) Chen, Y.; Zhang, J.; Luo, J.; Tu, J. M.; Zeng, X. L.; Xie, J.; Zhou, M.; Zhao, J. Q.; Scheer, H.; Zhao, K. H. Photophysical Diversity of Two Novel Cyanobacteriochromes with Phycocyanobilin Chromophores: Photochemistry and Dark Reversion Kinetics. *FEBS J.* **2012**, *279* (1), 40–54. https://doi.org/10.1111/j.1742-4658.2011.08397.x.
(3) Wiebeler, C.; Rao, A. G.; Gärtner, W.; Schapiro, I. The Effective Conjugation Length Is Responsible for the Red/Green Spectral Tuning in the Cyanobacteriochrome Slr1393g3. *Angew. Chemie - Int. Ed.* **2019**, *58* (7), 1934–1938. https://doi.org/10.1002/anie.201810266.

> P142. Poster

Symposium SENS-8 Cyanobacteriochromes. Characterization, Chromatic Adaptation and Applications in Optogenetics (J. Clark Lagarias)

PHOTOCONVERSION KINETICS OF A PHYTOCHROME LIKE CYANOBACTERIOCHROME GAF DOMAIN

Authors: Tobias Fischer[1], Qianzhao Xu[2,3], Alexander Gutt[4], Kai-Hong Zhao[3], Wolfgang Gärtner[2], Josef Wachtveitl[1], Chavdar Slavov[1]
Presenting Author: Tobias Fischer
1) Inst. Physical and Theoretical Chemistry, Goethe University Frankfurt/Main, Germany 2) Inst. Analytical Chemistry, University of Leipzig, Germany 3) State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, PR. China 4) Max-Planck-Inst. Chem. Energy Conv., Mülheim/Ruhr, Germany

Cyanobacteriochromes (CBCRs) are a subfamily of bilin-binding photoreceptors similar to canonical phytochromes. Their spectral diversity, photochemical properties and potential application in optogenetics have raised remarkable scientific interest. CBCRs consist of tandem arrays of GAF domains where chromophore binding and photoconversion are accomplished with a single GAF domain while in phytochromes the whole PAS-GAF-PHY triad is required.

All2699 (*Nostoc sp.* PCC 7120) is composed of three GAF domains and a signaling histidine kinase motif. Both GAF1 and GAF3 carry a phycocyanobilin chromophore and were characterized by steady state spectroscopy.[1]

Interestingly, similar to canonical phytochromes, All2699g1 undergoes photoconversion between a red-absorbing dark state (P_R, 637 nm) and a far-red-absorbing signaling state (P_{FR}, 690 nm), whereas most other CBCR GAF domains show a red-green switching cycle. We investigated the $P_R \leftrightarrow P_{FR}$ photodynamics by femtosecond transient absorption, FTIR spectroscopy and flash photolysis. The data were analyzed by lifetime density analysis (LDA) using the software OPTIMUS.[2]

The excited state dynamics of P_R* is significantly slowed down as compared to canonical phytochromes. P_R* relaxes to the ground state predominantly on the 100–1000 ps timescale to form the primary photointermediate LumiR. The kinetics is wavelength independent but is described by a relatively broad lifetime distribution. It follows the early reorganizition in the protein matrix instead of being distinctly heterogeneous as observed for other CBCRs.[3] The final photoproduct P_{FR} is subsequently formed on the ms timescale via a blue shifted Meta-R intermediate. In contrast to the P_R to P_{FR} transition dynamics, the P_{FR} to P_R reaction is similar to canonical phytochromes. The primary photoproduct is formed within 1-20 ps, possibly via several relaxation pathways. Spectral shifts on the ms timescale are indicative of protein reorganization in proximity of the chromophore.

Acknowledgements
C.S. and J.W. acknowledge DFG (WA 1850/4-2), K.H.Z. acknowledges the National Natural Science Foundation of China (31861143029 and 31770822).

References

[1] Chen, Y. et al., *The FEBS J.* **2012,** *279* (1), 40.
[2] Slavov, C. et al., *Anal. Chem.* **2015,** *87* (4), 2328.
[3] Kim, P. W. et al., *Biochemistry* **2012,** *51* (2), 608.

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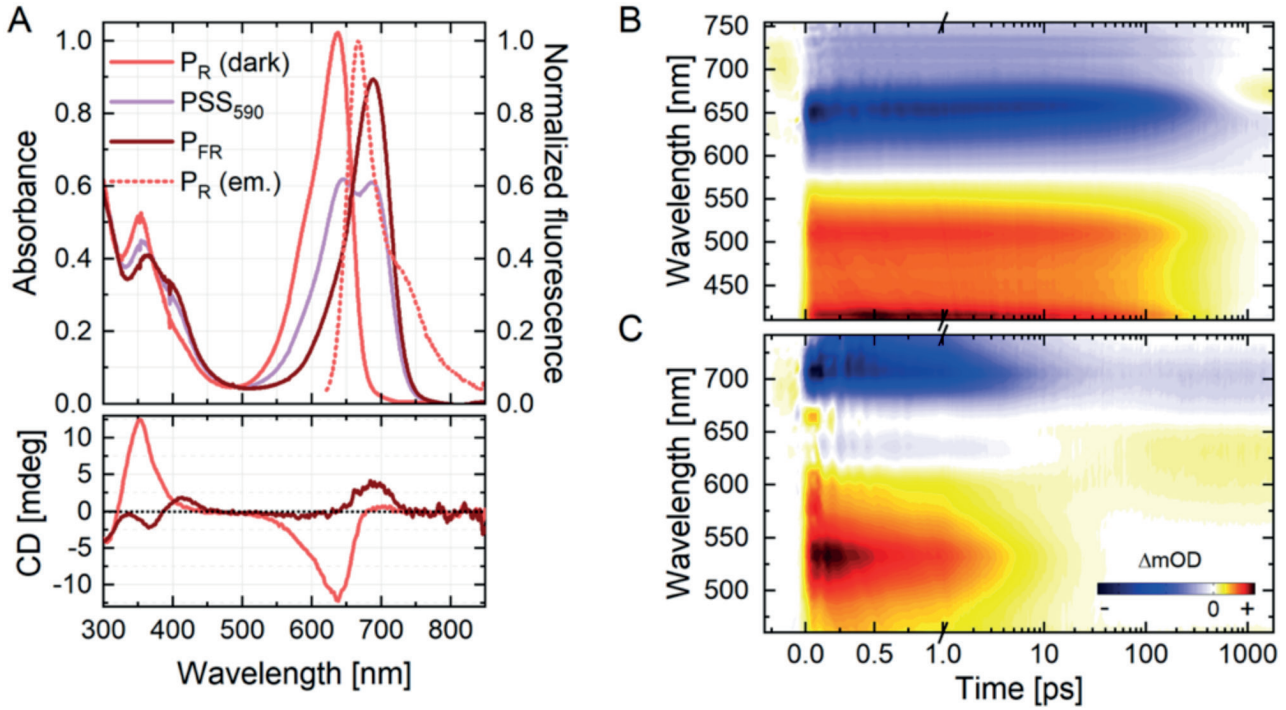

Fig. 1. A) UV/vis absorption and CD spectra of P_R and P_{FR}, fluorescence spectrum of P_R; B) Transient absorption data of P_R after 635 nm excitation; C) Transient absorption changes of P_{FR} after 720 nm excitation.

> IL298. Invited Lecture

Symposium SENS-9 Time-Resolved Serial Crystallography of Light-Sensitive Proteins (Sebastian Westenhoff)

PHOTOACTIVATION OF BACTERIAL PHYTOCHROMES STUDIED BY TIME-RESOLVED CRYSTALLOGRAPHY

Authors: Sebastian Westenhoff[1]
Presenting Author: Sebastian Westenhoff
1) Department of Chemistry and Molecular Boilogy, University of Gothenburg

Eye-less species use photosensor proteins to collect information about ambient light conditions. Phytochromes are a photosensor superfamily in plants, fungi, bacteria. Upon photoactivation of a biliverdin cofactor, the chromophore and protein undergo a series of structural changes on multiple time- and length scales in order alter the biochemical output activity. The structures of the resting and light-activated states of bacteriophytochromes are known, but the structural mechanism with which light cues are transferred into structural rearrangements are not well understood. In particular, the primary structural response of the chromophore and the surrounding residues remains elusive.

Here, we present a crystallographic investigation of the photoresponse the phytochrome from *D. Radiodurans*. We present a new room-temperature structure obtained by serial femtosecond X-ray crystallography at the Japanese X-ray free electron laser. I will also discuss time-resolved snapshots of the protein and its implication for the primary photoresponse of phytochrome proteins.

> IL299. Invited Lecture

Symposium SENS-9 Time-Resolved Serial Crystallography of Light-Sensitive Proteins (Sebastian Westenhoff)

OBSERVE WHILE IT HAPPENS: CATCHING PHOTORECEPTORS IN THE ACT WITH FREE ELECTRON LASERS AND COMPUTER SIMULATIONS

Authors: Gerrit Groenhof[1], Dmitry Morozov[1], Vaibhav Modi[1]
Presenting Author: Gerrit Groenhof
1) NanoScience Center & Department of Chemistry, University of Jyväskylä, Finland

Photochemistry is at the core of technologies for harvesting, converting and storing solar energy, but there are no good catalysts available that can steer the excited-state dynamics toward the desired product state while suppressing side reactions. So far, only Nature has evolved efficient ways to control the outcome of photochemical reactions, with vision and photosynthesis as prominent examples. Exploiting the principles of photobiology, however, requires a complete understanding of the underlying molecular dynamics. Before free electron lasers became available, the relevant time and spatial resolutions were notoriously difficult to access experimentally and much of our current understanding of the ultra-fast photo-dynamics in biological systems has been obtained with computer simulations. While serial femto-second time-resolved X-ray crystallography at free electron lasers has now opened up an experimental window into this regime, the current limitations of this technique still call for results from computer simulations to complement the experiments sometimes. In the talk, we will focus on recent applications in which we combined time-resolved X-ray diffraction with computational modeling to acquire atomistic insights into the activation mechanism of biological photoreceptors.

> IL300. Invited Lecture

Symposium SENS-9 Time-Resolved Serial Crystallography of Light-Sensitive Proteins (Sebastian Westenhoff)

ON RETINAL ISOMERIZATION IN BACTERIORHODOPSIN

Authors: Ilme Schlichting[1]
Presenting Author: Ilme Schlichting
1) Max Planck Institute for Medical Research

Bacteriorhodopsin (bR) is a light-driven proton pump. The primary photochemical event upon light absorption is isomerization of the retinal chromophore. We used time-resolved crystallography at an X-ray free-electron laser to follow the structural changes in multiphoton-excited bR from 250 femtoseconds to 10 picoseconds. Quantum chemistry and ultrafast spectroscopy were used to identify a sequential two-photon absorption process, leading to excitation of a tryptophan residue flanking the retinal chromophore, as a first manifestation of multiphoton effects. We resolve distinct stages in the structural dynamics of the all-trans retinal in photoexcited bR to a highly twisted 13-cis conformation. Other active site sub-picosecond rearrangements include correlated vibrational motions of the electronically excited retinal chromophore, the surrounding amino acids and water molecules as well as their hydrogen bonding network. These results show that this extended photo-active network forms an electronically and vibrationally coupled system in bR, and most likely in all retinal proteins

> IL301. Invited Lecture

Symposium SENS-9 Time-Resolved Serial Crystallography of Light-Sensitive Proteins (Sebastian Westenhoff)

A MOLECULAR MOVIE OF STRUCTURAL CHANGES IN THE LIGHT-DRIVEN PROTON PUMP BACTERIORHODOPSIN

Authors: Eriko Nango[1,2], So Iwata[1,2]
Presenting Author: Eriko Nango
1) Department of Cell Biology, Graduate School of Medicine, Kyoto University 2) RIKEN SPring-8 Center

Bacteriorhodopsin (bR) is a light-driven proton pump derived from *Halobacterium salinarum* and harvests the energy content of light to drive conformational changes leading to unidirectional proton transport. bR contains a buried all-trans retinal chromophore that is covalently bound to Lys216. The all-trans retinal undergoes isomerization to the 13-cis configuration by light absorption, initiating a photo-cycle and creating a sequence of spectral and structural changes. Considerable research has been devoted to understanding how structural changes in bR can transport a proton against a transmembrane potential. Many research groups have performed cryo-trapping experiments of bR using synchrotron radiation sources, thereby providing information on structural changes during the photo-cycle. Despite these successes, the experiments suffered from a number of weaknesses. Intermediate trapping studies were performed at low temperatures and thus were not truly time-dependent. Furthermore, conventional crystallography is subject to radiation damage so early results have been criticized.

We circumvented these concerns by recording a three-dimensional movie of structural changes in bR at room-temperature at 2.1 Å resolution using time-resolved (TR) serial femtosecond crystallography (SFX) at the SPring-8 Angstrom Compact Free Electron Laser (SACLA). The recent advent of intense, femtosecond X-ray pulses from X-ray free-electron laser (XFEL) has enabled the acquisition of diffraction patterns from protein microcrystals before the onset of radiation damage. In the TR-SFX experiment, a continuous stream of microcrystals was injected across a focused XFEL beam and the delay between sample photo-activation and the arrival of an XFEL pulse was controlled electronically.

TR-SFX data were collected from light-adapted bR microcrystals following photo-activation with a nanosecond laser pulse for Δt = 16 ns, 40 ns, 110 ns, 290 ns, 760 ns, 2 ms, 5.25 ms, 13.8 ms, 36.2 ms, 95.2 ms, 250 ms, 657 ms, and 1.725 ms (Fig.1). Our data revealed that an initially twisted retinal displaced Trp182 and Leu93 toward the cytoplasm and allowed a water molecule (Wat452) to order between Leu93, Thr89 and the Schiff base (SB) on the retinal in the L-state. Hydrogen-bonding interactions from the protonated SB, a proton donor to Wat452 and Thr89, created a pathway for proton transfer to a proton acceptor, Asp85. This observation explains how the SB makes contact with Asp85 despite being turned toward the cytoplasmic side by photo-isomerization. Once a proton was transferred, the hydrogen-bonding interaction between Asp85 and Thr89 was lost, which in turn interrupted connectivity to the extracellular side of the protein. The resulting cascade of structural changes throughout the protein provided unprecedented insights into how structural changes in bR conspire to achieve unidirectional proton transport.

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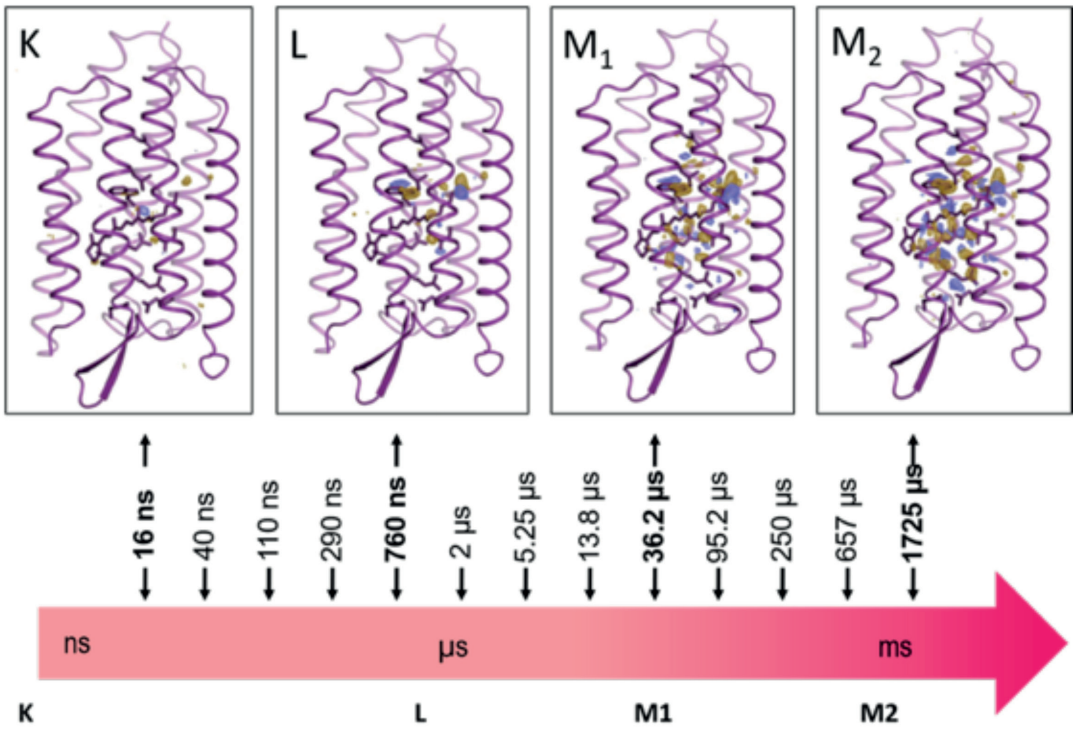
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> IL302. Invited Lecture

Symposium SENS-9 Time-Resolved Serial Crystallography of Light-Sensitive Proteins (Sebastian Westenhoff)

STRUCTURAL CHANGES OF OXYGEN-EVOLVING PSII DURING S-STATE TRANSITIONS AND A POSSIBLE MECHANISM FOR OXYGEN EVOLVING REACTION REVEALED BY X-RAY FREE LASER PULSES

Authors: Michi Suga[1,2], Jian-Ren Shen[1]
Presenting Author: Michi Suga
1) Okayama University 2) JST, PREST

Introduction

Photosynthetic water oxidation is catalyzed by the Mn_4CaO_5-cluster[1,2] of Photosystem II (PSII) through four steps of oxidation via the Si-state cycle (S_i, i = 0-4). The catalyst becomes a Mn_4CaO_6-cluster in the S_3-state by incorporation of an additional oxygen O6 nearby a unique central oxo-bridge O5[3], and therefore the roles of O5 and O6 during the O=O bond formation has been discussed. While insertion of the O6 has gradually been accepted, the chemical structure of O5 and O6 remain controversial so that several possible mechanisms for the O=O bond formation (for instance, an oxo/oxyl radical coupling mechanism, a nucleophilic attack reaction mechanism, and an intermediate peroxide mechanism) have been under debate, even though the XFEL structures greatly narrowed the possible mechanisms. To reveal the molecular details in the water oxidation reaction, we analyzed the X-ray free laser (XFEL) structures of PSII in the intermediate S_2 and S_3 states at atomic resolutions.

Results

In the S_2 state, the Mn_4CaO_5-cluster shows the canonical open-cubane structure. Upon transition to the S_3 state, flipping of D1-Glu-189 provides a space for incorporation of the additional oxygen O6, and the Mn_4CaO_5-cluster remains in the open-cubane form. We carefully examined possible chemical structures of the O5 and O6 atoms by analyzing the previous and new datasets. Structural analysis is still ongoing and I will show detailed results of the structural analysis, which provide structural insights into how structural changes induced by flash illuminations enabled the substrate water access, proton release, and O=O bond formation in PSII.

Acknowledgments

We thank many collaborators who are not listed here due to the limited space. This work was supported by JSPS KAKENHI and JST, PREST. Diffraction data were collected at beamline three of SACLA with the approval of JASRI.

References

1 Umena, Y. et. al. Nature 473, 55-60, (2011).
2 Suga, M. et al. Nature 517, 99-103, (2015).
3 Suga, M. et al. Nature 543, 131-135, (2017).

> IL303. Invited Lecture

Symposium SENS-9 Time-Resolved Serial Crystallography of Light-Sensitive Proteins (Sebastian Westenhoff)

WATER OXIDATION REACTION OF PHOTOSYSTEM II STUDIED USING SIMULTANEOUS X-RAY CRYSTALLOGRAPHY AND SPECTROSCOPY AT XFELS

Authors: Vittal Yachandra[1], Ruchira Chatterjee[1], Mohamed Ibrahim[2], Louise Lassalle[1], Thomas Fransson[3], Aaron Brewster[1], Iris Young[1], Rana Hussein[2], Miao Zhang[2], Franklin Fuller[3], Sheraz Gul[1], Casper de Lichtenberg[4], Mun Hon Cheah[4], Roberto Alonso-Mori[3], Uwe Bergmann[3], Nicholas Sauter[1], Athina Zouni[2], Johaness Messinger[4], Jan Kern[1], Junko Yano[1]
Presenting Author: Vittal Yachandra
1) Lawrence Berkeley National Laboratory, Berkeley, CA, USA 2) Institut für Biologie, Humboldt-Universität zu Berlin, Berlin, Germany 3) SLAC National Accelerator Laboratory, Menlo Park, CA, USA 4) Department of Chemistry, Molecular Biomimetics, Uppsala University, Uppsala, Sweden.

The development of XFELs has opened up opportunities for studying the dynamics of biological systems beyond what is possible at synchrotron radiation sources. Intense XFEL pulses enable us to apply both X-ray diffraction and spectroscopic techniques to dilute systems or small protein crystals. By taking advantage of ultra-bright femtosecond X-ray pulses, one can also collect the data under functional conditions of temperature and pressure, in a time-resolved manner, after initiating reactions, and follow the chemical dynamics during catalytic reactions and electron transfer. Such an approach is particularly beneficial for biological materials and aqueous solution samples that are susceptible to X-ray radiation damage.

We have developed spectroscopy and diffraction techniques necessary to fully utilize the capability of the XFEL X-rays for a wide-variety of metalloenzymes, like Photosystem II (PS II), and to study their chemistry under functional conditions (room temperature, ambient pressure). One of such methods is simultaneous data collection for X-ray crystallography and X-ray spectroscopy, to determine the overall structural changes of the protein and chemical changes at metal catalytic sites, as the enzyme advances through the catalytic cycle in real time under ambient conditions. The other method is soft X-ray absorption spectroscopy of metalloenzymes by developing a spectrometer capable of studying dilute biological systems under ambient conditions.

We have used the above techniques to study the water oxidation reaction of PS II, a multi-subunit protein complex, in which the Mn_4CaO_5 cluster catalyzes the reaction. The current status of this research and the mechanistic understanding of the water oxidation reaction in PS II based on the XFEL based X-ray techniques will be presented.

References

[1] F. D. Fuller *et al.*, Nature Methods **14**, 443-449 (2017)
[2] I. D. Young *et al.*, Nature **540**, 453 (2016)
[3] M. Kubin *et al.*, Struct. Dyn. **4**, 054307 (2017)
[4] J. Kern *et al.*, Nature **563**, 421 (2018)

> IL304. Invited Lecture

Symposium SENS-9 Time-Resolved Serial Crystallography of Light-Sensitive Proteins (Sebastian Westenhoff)

FEMTOSECOND X-RAY EXPERIMENTS AT EUROPEAN XFEL

Authors: Wojciech Gawelda[1], Andreas Galler[1], Christian Bressler[1]
Presenting Author: Christian Bressler
1) European XFEL

Time-resolved x-ray tools allow measuring electronic and geometric structure changes. X-Ray emission spectroscopy is sensitive to electronic changes, such as oxidation and spin states, while x-ray absorption fine structure tools deliver information about the local geometric structure around the selected absorbing atom. Combining these tools with forward scattering in one single setup allows extracting simultaneous information about the local to rather global structural changes occurring in the reacting system.

We will present some case examples, for which pico- and femtosecond x-ray experiments deliver new insight into evolving dynamic processes, including reactive high-valent iron compounds and a class of spin transition systems. This will be preceded by an introduction about the information content of x-ray tools.

Finally, all these tools can be combined into one single experimental setup, and the Femtosecond X-Ray Experiments (FXE) Instrument at European XFEL will allow just this, and its operation just started in late summer 2017. We will present the current status of this new instrument at European XFEL [1,2] together with some early results.

References

[1] *Photon Beam Transport and Scientific Instruments at the European XFEL* T. Tschentscher, C. Bressler, J. Grünert, A. Madsen, A. P. Mancuso, M. Meyer,A. Scherz, H. Sinn, U. Zastrau, Appl. Sci. **7**, 592 (2017)
[2] *Femtosecond X-Ray Experiments (FXE): Instrumentation and Baseline Experimental Capabilities*. A. Galler, W. Gawelda, *et al.*, J. Synchr. Rad., in press (2019)

> IL310. Invited Lecture

Symposium SENS-10 Cryptochromes. Mechanisms. Magnetoreception and UV Light (Margaret Ahmad)

PHOTORECEPTOR SIGNALING CROSSTALK IN THE REGULATION OF PLANT RESPONSES TO THE ENVIRONMENT

Authors: Elena Monte[1]
Presenting Author: Elena Monte
1) CRAG - Centre for Research in Agricultural Genomics

Light is not only an energy source for plants but also a signal that informs them about their environment. Because they cannot move very far, plants have mechanisms to integrate these light signals and adapt to changes in their surroundings. Light intensity, quality, direction and duration impact plant growth and development during the whole life span, from seed germination to flowering. In Arabidopsis seedlings, the blue light-sensing cryptochromes (crys) and red/far-red light-sensing phytochromes (phys) play critical roles in mediating light regulation of hypocotyl elongation, cotyledon expansion, pigment accumulation, stomata development and opening, and light entrainment of the circadian clock. Recent data have shown that cry and phy signaling are integrated and mediate transcriptomic changes that affect more than 10% of the Arabidopsis genome. This crosstalk involves the interaction with common transcription factors of the bZIP and bHLH families. Cry and phy signaling integration allows plants to respond to environmental challenges with plasticity for optimal fitness. This will be illustrated by discussing novel data on stomata dynamics.

> IL309. Invited Lecture

Symposium SENS-10 Cryptochromes. Mechanisms. Magnetoreception and UV Light (Margaret Ahmad)

MOLECULAR MECHANISMS OF HUMAN SLEEP TIMING

Authors: Alina Patke[1]
Presenting Author: Alina Patke
1) The Rockefeller University

Patterns of daily human activity are controlled by an intrinsic circadian clock that promotes ~24 hr rhythms in many behavioral and physiological pro- cesses. This system is altered in delayed sleep phase disorder (DSPD), a common form of insomnia in which sleep episodes are shifted to later times mis- aligned with the societal norm. Here, we report a he-reditary form of DSPD associated with a dominant coding variation in the core circadian clock gene CRY1, which creates a transcriptional inhibitor with enhanced affinity for circadian activator proteins Clock and Bmal1. This gain-of-function CRY1 variant causes reduced expression of key transcriptional targets and lengthens the period of circadian molec- ular rhythms, providing a mechanistic link to DSPD symptoms. The allele has a frequency of up to 0.6%, and reverse phenotyping of unrelated families corroborates late and/or fragmented sleep patterns in carriers, suggesting that it affects sleep behavior in a sizeable portion of the human population.

> IL308. Invited Lecture

Symposium SENS-10 Cryptochromes. Mechanisms. Magnetoreception and UV Light (Margaret Ahmad)

COMBINED MAGNETIC AND LIGHT EFFECTS ON CRYPTOCHROME ACTIVITY

Authors: Phillise Todd[1], Shawn Strausser[1], Thorsten Ritz[1]
Presenting Author: Thorsten Ritz
1) UC Irvine

Cryptochromes are blue-green light photoreceptors involved in regulating a wide range of responses, from plant growth to entraining the circadian clock in insects. During photoactivation, the active co-factor FAD is reduced under blue-green light to a semiquinone form that can be further reduced under longer wavelength light to a fully reduced form. Reoxidation occurs in darkness. Cryptochromes have also been suggested as potential magnetoreceptors with the magnetic fields affecting either the forward photoactivation reaction or the back-reoxidation reaction via their effects on radical-pairs formed during the photocycle reaction steps. In this model, the magnetic field effects would become manifest as an indirect effect on light sensing. That is, an increase in magnetic field intensity would lead to an effect on Cry responses comparable to that of a change in light intensity. Indeed, using Cry phosphorylation as a measure of Cry activity, it was shown that MFs enhance Cry activity in a manner generally consistent with expectation from the radical-pair mechanism. Here, we provide a model quantifying the combined magnetic and light effects on Cry by combining kinetic modeling of the photocycle with a simple signal transduction model. We apply this model to compare results from bird magnetic orientation experiments in different light conditions with expectations from a radical-pair based compass based on Cry responses. We will discuss important qualitative features and strategies to find particular diagnostic conditions for combined light and magnetic field effects on Cry responses.

> IL311. Invited Lecture

Symposium SENS-10 Cryptochromes. Mechanisms. Magnetoreception and UV Light (Margaret Ahmad)

THE MAGNETIC COMPASS OF BIRDS

Authors: Roswitha Wiltschko[1], Wolfgang Wiltschko[1]
Presenting Author: Roswitha Wiltschko
1) Goethe-Universtät Frankfurt a.M., Germany

Birds can use the geomagnetic field for compass orientation. This was first demenstrated basec on the migratoy orientation of a small songbird, the European Robin: In a cage, the birds preferred their naturalmigratory direction, but when magnetic North was deflected by Helmholtz coils, the birds shifted their headings accordingly. Further analysis of the avian magnetic compass revealed unexpected propertiers: (1) it is not sensitive to the polarity of the magnetic field, but only senses the (axial) course of the field lines; (2) it functiones only in a "biological window" around the intensity the birds a used to, and (3) it requires short-wavelength light from UV to green. These properties caused Ritz and colleagues in 2000 to propose the Radical-Pair-Model of magnetoreception, with cryptochrome as receptor molecule forming the radical pairs and the eyes as site of magnetoreception. Cryptochromes were indeed found in cone cells in the retina of birds; they are activated by light, Behavioral experiments suggest that the crucial radical pairs are formed during reoxidation.

References

Wiltschko, R., Wiltschko, W. (2014): Biosensors 4, 221-241.
Wiltschko, R. et al. (2016): J. R. Soc. Interface 13, 20151010

> IL305. Invited Lecture

Symposium SENS-10 Cryptochromes. Mechanisms. Magnetoreception and UV Light (Margaret Ahmad)

PLANT CRYPTOCHROME: MECHANISM OF PHOTORECEPTION FROM INFRARED SPECTROSCOPY

Authors: Lea Schroeder[1], Lena Bögeholz[1], Tilman Kottke[1]
Presenting Author: Tilman Kottke
1) Physical and Biophysical Chemistry, Bielefeld University

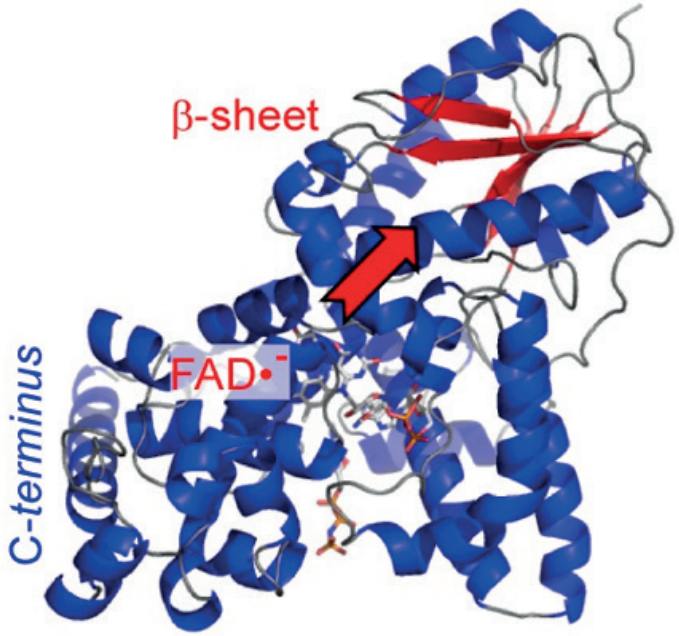
Cryptochromes are blue light receptors regulating plant growth and daily rhythm and acting as magnetoreceptors in insects [1]. Blue light absorption by oxidized flavin in the sensory photolyase homology region (PHR) comprising ~500 amino acids leads to ultrafast electron transfer to the flavin chromophore. We have investigated the subsequent steps in the PHR of plant cryptochrome (pCRY) from *Chlamydomonas reinhardtii* by time-resolved visible and infrared spectroscopy. The analysis revealed the protonation of flavin by a nearby aspartic acid (D396) within few microseconds. Subsequently, the b-sheet at a distance of ~25 Å to the flavin is reorganized (see Figure) [2].

One critical difference between plant and insect cryptochromes is the proton transfer to flavin, which only occurs in plant cryptochromes. We introduced a corresponding mutation in pCRY (D396C) and demonstrate that, strikingly, the b-sheet response is preserved, even with a similar time constant of about 1 ms [3]. Therefore, the decisive event for driving structural changes is the formation of a charged flavin radical in a hydrophobic pocket, which takes place in both plant and insect cryptochromes.

We further applied in-cell infrared spectroscopy and found an influence of cytosolic nucleotides on the light-driven structural reorganization of pCRY in intact bacterial cells. Similarities and differences between results *in vitro* and in cells will be discussed.

References

[1] Chaves, I., Pokorny, R., Byrdin, M., Hoang, N., Ritz, T., Brettel, K., Essen, L. O., van der Horst, G. T., Batschauer, A., and Ahmad, M. (2011) *Annu. Rev. Plant Biol. 62*, 335.
[2] Thöing, C., Oldemeyer, S., and Kottke, T. (2015) *J. Am. Chem. Soc. 137*, 5990.
[3] Schroeder, L., Oldemeyer, S., and Kottke, T. (2018) *J. Phys. Chem. A 122*, 140.



> IL306. Invited Lecture

Symposium SENS-10 Cryptochromes. Mechanisms. Magnetoreception and UV Light (Margaret Ahmad)

CIRCADIAN CLOCK PHOTOENTRAINMENT BY DROSOPHILA CRYPTOCHROME

Authors: Brian R Crane[1]
Presenting Author: Brian R Crane
1) Cornell University

Cryptochromes (CRYs) are blue-light sensors that play key roles in the circadian clocks of plants and animals. These proteins consist of a highly conserved photolyase homology region (PHR) that binds the flavin cofactor FAD and a C-terminal tail extension (CTE) of varying size. In CRY from the fruit fly *Drosophilamelanogaster* (dCRY), photoreduction of FAD triggers changes in protonation state and conformation, including the undocking of a C-terminal Tail (CTT) helix. The CTT gates interactions with targets of dCRY light-activation: the proteins Timeless (TIM) and Jetlag (JET).

Four conserved tryptophan residues (W420, W397, W342, W394) mediate electron transfer for flavin photoreduction of dCRY. Substitutions of these residues to both redox-inert phenylalanine residues and redox-active tyrosine residues affect dCRY light sensitivity. FAD photoreduction yields of the variants correlate well with the extent of conformational change at the CTT and biological activity. The surface residue W394 is the most indispensable for some functions and its relocation can produce variant dCRYs more sensitive to light than the WT. Residue substitutions in the flavin-binding pocket alter the redox couple of the flavin cofactor driven by light and thereby tune the spectral response.

DCRY interacts with TIM and JET in a light depend-manner to entrain the circadian clock to environmental cues. In response to light, dCRY initiates both TIM degradation and its own proteolysis. Residue substitutions that affect the flavin photoreduction process have differential impacts on these two activities, thereby providing a means to separate biological functions. However, how light-driven conformational changes within dCRY gate the molecular interactions among dCRY and its partners is not well understood. Approaches to map the interfaces among these transient complexes and stabilize their assemblies for structural studies will be presented.

> IL307. Invited Lecture

Symposium SENS-10 Cryptochromes. Mechanisms. Magnetoreception and UV Light (Margaret Ahmad)

CRYPTOCHROME PHOTORECEPTOR MECHANISM OF ACTIVATION AND RESPONSIVITY TO ELECTROMAGNETIC FIELDS.

Authors: Margaret Ahmad[1,2], Nathalie Jourdan[1], Louis-David Arthaut[1], Marootpong Pooam[1], Maria Procopio[3], Carlos Martino[4]
Presenting Author: Margaret Ahmad
1) Sorbonne University 2) Xavier University 3) Johns Hopkins University 4) Florida Institute of Technology

Cryptochromes are evolutionarily conserved flavoprotein receptors with diverse physiological functions in organisms ranging from plants to humans. Cryptochromes undergo a photocycle involving flavin photoreduction and the biosynthesis of ROS. Recently, cryptochromes have been proposed as possible magnetosensors based on their photochemical properties, which are compatible with the Radical Pair Model of chemical magnetosensing. This suggestion is supported by increasing evidence of cryptochromes implicated in magnetosensing in multiple organisms.

In this presentation we provide additional evidence for the mechanism whereby redox change in the *Arabidopsis* cryptochrome triggered by light achieves the final outcome of a conformational change linked to downstream signaling pathways. Phosphorylation at the C-terminal domain is evaluated in wild type and mutant proteins having altered flavin photochemistry, showing correlation of conformational change with flavin reduction. The cryptochrome photocycle is linked to its role as a possible magnetoreceptor by identifying steps in the redox cycle that may form unpaired radicals as predicted by the Radical Pair Model. These effects are evaluated at both low level (near-zero) and 10 fold earth strength (500 **μ**T) static magnetic fields. In further support of this model, we show that the response of cryptochrome to the earth's magnetic field is disrupted by RF (radiofrequency) signals in the 7 MHz frequency range, analogous to observations in migratory birds. Theoretical and kinetic studies show that direct magnetosensing by cryptochrome likely occurs by enhanced quantum yield of the flavin reoxidation reaction and thereby obeys many of the characteristics of the Radical Pair Model.

A coherent model of the possible plant cryptochrome magnetoreception mechanism will be discussed.

> P143. Poster

Symposium SENS-10 Cryptochromes. Mechanisms. Magnetoreception and UV Light (Margaret Ahmad)

QUANTUM CASCADE LASER SETUP FOR TIME-RESOLVED INFRARED SPECTROSCOPY ON IRREVERSIBLE CRYPTOCHROME PHOTOREACTIONS

Authors: Jessica Laura Klocke[1], Tilman Kottke[1]
Presenting Author: Jessica Laura Klocke
1) Bielefeld University

Plant cryptochromes are blue light photoreceptors binding flavin as a chromophore. They regulate central aspects of plant and algal growth and development. A valuable tool to investigate the complex mechanism and analyze changes in the secondary and tertiary structure of the photoreceptors is time-resolved vibrational spectroscopy [1]. However, irreversible processes are challenging to study, particularly in H_2O [2], requiring the application of a flow cell and thus excessive amounts of sample.

Here, we present a setup based on a broadly tunable quantum cascade laser (QCL) with a spectral range of 1740-1495 cm^{-1}. We recorded kinetics over a broad time range with 30 ns time resolution of the irreversible photoreduction of flavin by EDTA in H_2O. In contrast to step-scan FTIR experiments [2], the sample consumption can be drastically reduced by the possibility to focus the infrared beam to the diffraction limit at much higher probe light intensity. To further reduce the sample consumption, a micrometer stage moves the flow cell perpendicular to the flow direction after each excitation of the sample.

The setup is applied to study the photocycle of plant cryptochrome with nanosecond time resolution. This includes investigations in the presence of ATP, which renders the photoreaction irreversible. We will further extend this approach to other cryptochromes, such as the animal-like cryptochrome (aCRY) from *Chlamydomonas reinhardtii*. The high time resolution and minimal sample consumption of the setup will be exploited to analyze changes in the secondary structure of aCRY in response to red light.

References

[1] C. Thöing, S. Oldemeyer, T. Kottke, 2015, *J. Am. Chem. Soc. 137*, 5990-5999.
[2] C. Thöing, A. Pfeifer, S. Kakorin, T. Kottke, 2013, *Phys. Chem. Chem. Phys. 15*, 5916–5926.

> OC113. Oral Communication
Symposium SENS-11 Short Communications on Photosensory Biology (Silvia Braslavsky)

TOWARD PHOTODYNAMIC PHYSIOLOGY OF CHOLECYSTOKININ 1 RECEPTOR WITH GENETICALLY ENCODED PROTEIN PHOTOSENSITISER MINISOG

Authors: Yuan Li[1], Zong Jie Cui[1]
Presenting Author: Zong Jie Cui
1) Beijing Normal University

Cholecystokinin 1 receptor (CCK1R) is permanently activated by singlet oxygen ($^1O_2$) which is generated in a type II photodynamic action. To make this novel property amenable for wider applications, we have fused genetically encoded protein photosensitizer miniSOG to the CCK1R sequence and examined photodynamic CCK1R activation by Fura-2 calcium imaging in transfected cell lines. Light irradiation (450 nm, 85 mW.cm^{-2}, 90 s) of pancreatic acinar cell AR4-2J expressing miniSOG$_{mem}$ triggered calcium oscillations blockable by CCK1R antagonist devazepide 2 nM. miniSOG fused to N- or C-terminus of CCK1R (miniSOG-CCK1R, CCK1R-miniSOG) retained the ability to photodynamically activate the in-frame CCK1R. Linker (GlySerGly)$_n$ insertion between miniSOG and CCK1R [miniSOG-(GSG)$_n$-CCK1R] was tolerated with (GlySerGly)$_{4,8}$ but not with (GlySerGly)$_{12}$. Addition of an IRES sequence (miniSOG-IRES-CCK1R), however, did not result in effective photodynamic activation of in-frame CCK1R. $^1O_2$ quencher uric acid (50 microM, 1 mM) or Trolox-C (300 microM) completely inhibited miniSOG photodynamic CCK1R activation. miniSOG fusion with NanoLuc (miniSOG$_{mem}$-IRES-NanoLuc) provided sufficient bioluminescence light with substrate coelenterazine to power miniSOG photodynamic activation of CCK1R in AR4-2J. Barrel-structured KillerRed was similarly effective when fused either to the N- or the C-terminus of CCK1R (KillerRed-CCK1R or CCK1R-KillerRed). The above data together indicate that permanent photodynamic activation of CCK1R is achieved after fusion of genetically encoded photosensitisers with CCK1R. The present work provides a novel toolkit to study the physiology of CCK1R-expressing cells and tissues. Extension of this property further may lead to a full spectrum of toolkits for the study of other G protein-coupled receptors.

Keywords
miniSOG, CCK1R, calcium oscillations, NanoLuc, KillerRed, AR4-2J

Funding
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References
Jiang WY, Li Y, Li ZY, Cui ZJ (2018) *Cell Mol Neurobiol* **38**: 1283-1292.
Jiang HN, Li Y, Jiang WY, Cui ZJ (2018) *Front Physiol* **9**: 497.
Jiang HN, Li Y, Cui ZJ (2017) *Front Physiol* **8**: 191.

> OC114. Oral Communication

Symposium SENS-11 Short Communications on Photosensory Biology (Silvia Braslavsky)

MECHANISTIC STUDY OF FATTY ACID PHOTODECARBOXYLASE - A NEW PHOTOENZYME CONVERTING FATTY ACIDS TO ALKANES USING VISIBLE LIGHT

Authors: Pavel Müller[1], Klaus Brettel[1], Damien Sorigué[2], Poutoum-Palakiyem Samire[2], Frédéric Beisson[2]
Presenting Author: Pavel Müller
1) Institute for Integrative Biology of the Cell (I2BC), CEA Saclay, CNRS, University Paris-Sud, University Paris-Saclay, 91198 Gif-sur-Yvette, FRANCE 2) Biosciences and Biotechnologies Institute of Aix-Marseille (BIAM), CEA Cadarache, CNRS, Aix-Marseille University, 13108 Saint-Paul-lez-Durance, FRANCE

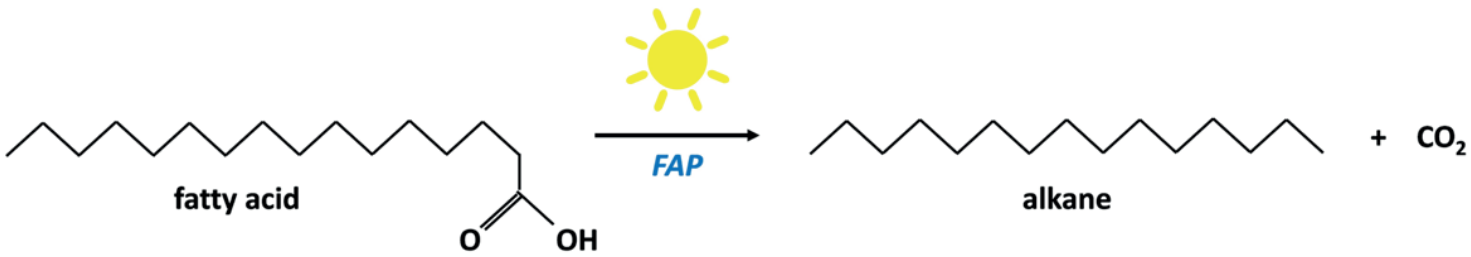
Single-cell green algae *Chlamydomonas reinhardtii* and *Chlorella variabilis* have been recently found to produce long-chain n-alkanes and n-alkenes in a light-dependent manner [1]. It turns out that these hydrocarbons are yielded by decarboxylation of the corresponding fatty acids and this reaction is catalysed by a novel type of photoenzyme belonging to the protein family of glucose-methanol-choline oxidoreductases. We have named the photoenzyme '*Fatty Acid Photodecarboxylase*' (*FAP*).

We have studied the mechanism of *FAP* function by means of time-resolved fluorescence and transient absorption spectroscopy [2]. *FAP* contains a non-covalently bound and fully oxidized flavin adenine dinucleotide cofactor (FAD), which absorbs UV & visible light up to ~530 nm. Situated < 4 Å from the carboxylate of the substrate (*i.e.*, the fatty acid: R-COO^-), the photoexcited FAD abstracts an electron from $RCOO^-$ within ~300 ps, generating a pair of radicals: $FAD^{\bullet-}$ and $RCOO^\bullet$. $RCOO^\bullet$ spontaneously decarboxylates, giving rise to an alkyl radical $R^\bullet$ and CO_2. The electron is then transferred back from $FAD^{\bullet-}$ to $R^\bullet$ within ~100 ns. This process is likely coupled to a transfer of a proton from a donor (XH), which is yet to be identified. The resulting alkane/alkene and CO_2 are replaced by a new substrate within a few tens of milliseconds.

The decarboxylation reaction was found to be highly efficient (>80% quantum yield) and with a turnover rate of >15 fatty acids to hydrocarbons per second, the *FAP* largely outperforms (at least 10-times) the hitherto known and used thermally activated decarboxylases.

The discovery of *FAP* opens a new avenue for a "green" production of fuel-like hydrocarbons from non-fossil sources and sets the stage for design and development of new flavin-based catalysts.

References
[1] Sorigué, D.; Légeret B.; Cuiné, S.; Morales, P.; Mirabella, B.; Guédeney, G.; Li-Beisson, Y.; Jetter, R.; Peltier, G.; Beisson, F. *Plant Physiol.*, **2016**, *171*, 2393–2405.
[2] Sorigué, D.; Légeret B.; Cuiné, S.; Blangy, S.; Moulin S.; Billon, E.; Richaud, P.; Brugière, S.; Couté, Y.; Nurizzo, D.; Müller, P.; Brettel, K.; Pignol, D.; Arnoux, P.; Li-Beisson, Y.; Peltier, G.; Beisson, F. *Science*, **2017**, *357*, 903-907.



> ON115. Oral Communication

Symposium SENS-11 Short Communications on Photosensory Biology (Silvia Braslavsky)

INTEGRATED OPTICAL CHARACTERIZATION OF LIGHT-SENSITIVE PROTEIN FILMS

Authors: Szilvia Krekic[1], Dávid Nagy[1], Stefka G. Taneva[2], László Fábián[1], Tomas Zakar[3], Zoltán Gombos[3], László Zimányi[1], András Dér[1]
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Integrated optics (IO) is a new discipline of science that proposes an alternative solution for demanding problems currently faced in integrated electronics. IO is analogue to integrated electronics but information transfer and data processing are done dominantly by optical means, which enables faster speed of operation, with less thermal effects arising in a miniature device. The bottleneck of IO is finding materials with suitable non-linear optical properties that can be used as active components in IO circuits, thus controlling the flow and rate of information transfer. Numerous organic and inorganic materials have been considered for this purpose, however none of them is deemed ideal. Our research group's main objective is to investigate the spectrokinetic properties of photochromic proteins for possible integrated optical applications.

In our current work, we investigated the optical properties of dried biofilms made of photoactive yellow protein (PYP) and phycobiliproteins (PBPs), comparing them to our previous experiments done with the protein bacteriorhodopsin (bR). The proteins' light-induced refractive index changes were measured using the Optical Waveguide Lightmode Spectroscopy method, while the spectral changes were monitored by an Optical Multichannel Analyzer. The measured data were analyzed using singular value decomposition, the number of intermediates were determined with global multiexponential fit, and a photocycle scheme was fitted to determine the kinetic properties for each intermediate.

Our results imply that the used protein films can be promising candidates for optical switching experiments, and combining them with passive IO devices they might be considered valid alternative options as active components in IO circuits.

Acknowledgements
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> OC116. Oral Communication

Symposium SENS-11 Short Communications on Photosensory Biology (Silvia Braslavsky)

INTERACTION BETWEEN TWO BACTERIOPHYTOCHROMES AND THEIR SIGNAL TRANSDUCTION IN AGROBACTERIUM FABRUM

Authors: Peng Xue[1], Afaf El Kurdi[1], Norbert Krauß[1], Tilman Lamparter[1]
Presenting Author: Peng Xue
1) Karlsruhe Institute of Technology

Agrobacterium fabrum is non-photosynthetic and gram-negative soil bacterium that causes crown gall (tumor) formation in at least 140 species of dicotyledons or gymnosperms. Tumor formation is initiated by a transfer of T-DNA from Ti plasmid of the bacterium into the genome of the plant cell. A. fabrum type IV secretion system (T4SS) and the type VI secretion system (T6SS) play an important role in the T-DNA transfer and bacterial competition, respectively. Previous results from our group showed that white light suppressed the infection of cucumber (Cucumis sativus) stems with A. fabrum [1]. In A. fabrum, there is one pair of phytochromes, Agp1 and Agp2 [2, 3]. The transfer of plasmid between cells is observed during bacterial conjugation. In A. fabrum, both phytochromes could regulate the conjugation [4].

We observed that growth rates of both single (agp1- or agp2-) and double (agp1/2-) mutants of A. fabrum were higher than that of wild type. We also used the same mutants of A. fabrum to infect the stems and leaves of Nicotiana benthamiana in darkness and red light at room temperature. Both A. fabrum phytochromes have a positive impact on plant infection. Red light inhibited the effect of Agp1 but promoted that of Agp2. We also performed competition assays with A. fabrum WT and double knockout mutant. We inoculated A. fabrum together with soil bacteria for a given time and obtained sequences of hypervariable region V2 of the 16S rRNA gene. In this way, the composition of bacteria was obtained. The phytochromes could improve the interbacterial competition.

In order to study possible interaction between Agp1 and Agp2, we performed measurements size exclusion, photoconversion, dark reversion, autophosphorylation and chromophore assembly kinetic measurements on Agp1/Agp2 mixtures. In all assays the data obtained from mixed samples were different from the (added) data obtained from single phytochromes. These assays showed therefore that Agp1 and Agp2 interact in vitro.

TMT-based quantitative proteomics results showed that in A. fabrum, phytochromes could regulate the haemolysin-coregulated protein and toxin protein Atu4347 of T6SS to control the interbacterial competition and also promote expression of conjugation protein TraA to affect the conjugation.

References

1. Oberpichler, I., Rosen, R., Rasouly, A., Vugman, M., Ron, E. Z. & Lamparter, T. (2008) Light affects motility and infectivity of Agrobacterium tumefaciens, Environmental microbiology. 10, 2020-2029.
2. Goodner, B., Hinkle, G., Gattung, S., Miller, N., Blanchard, M., Qurollo, B., Goldman, B. S., Cao, Y., Askenazi, M. & Halling, C. (2001) Genome sequence of the plant pathogen and biotechnology agent Agrobacterium tumefaciens C58, Science. 294, 2323-2328.
3. Wood, D. W., Setubal, J. C., Kaul, R., Monks, D. E., Kitajima, J. P., Okura, V. K., Zhou, Y., Chen, L., Wood, G. E. & Almeida, N. F. (2001) The genome of the natural genetic engineer Agrobacterium tumefaciens C58, science. 294, 2317-2323.
4. Bai, Y., Rottwinkel, G., Feng, J., Liu, Y. & Lamparter, T. (2016) Bacteriophytochromes control conjugation in Agrobacterium fabrum, Journal of Photochemistry and Photobiology B: Biology. 161, 192-199.

> OC117. Oral Communication

Symposium SENS-11 Short Communications on Photosensory Biology (Silvia Braslavsky)

PHYTOCHROME ACTION AND LIGHT-INDUCED SIGNAL TRANSDUCTION IN AGROBACTERIUM FABRUM

Authors: Afaf El Kurdi[1], Peng Xue[1], Tilman Lamparter[1], Norbert Krauß[1], Gernot Guigas[1], Ulrich Nienhaus[1]
Presenting Author: Afaf El Kurdi
1) Karlsruhe Institute of Technology

Phytochromes are biliprotein photoreceptors in plants, fungi and bacteria. They play an important role in plant growth, development and regulation of metabolic mechanisms in response to light.

Agrobacterium has two phytochrome proteins, Agp1 and Agp2 that exhibit opposite spectral characteristics and photoconversion between the red absorbing Pr form and the far-red absorbing Pfr form. Knockout and overexpression studies of these phytochromes showed that they work together and affect bacterial conjugation.

The crystal structures of phytochromes have revealed insight into protein conformational changes of the PCM region and details of the tongue. For instance, the long helix connecting the GAF and PHY domain is flexible in the Pr form and locked in a stretched conformation in the Pfr form, and the arm of the tongue undergoes secondary structure changes from b-sheet in Pr to α-helix in Pfr. Furthermore, PELDOR experiments before and after photoconversion were analysed, where the distance between both subunits of the Agp1 dimer at different positions of the protein was measured, showing no significant distance changes upon Pr-Pfr conversion.

We therefore aim to understand the complete signal transduction pathway of a bacterial phytochrome system involving the photoisomerization, light induced protein conformational changes and modulation of kinase activity.

Moreover, Agp1 is investigated by time resolved fluorescence anisotropy in order to gain insight into the dynamics of the protein to check if there are any dynamic changes during photoconversion. Different mutants were prepared to introduce one cysteine residue each at a definite position by site directed mutagenesis, enabling targeted labeling with Atto565 fluorophores via their maleimide groups. First results have shown possible changes in Agp1 flexibility between Pr and Pfr. The chosen mutation positions were identical to the ones used in the PELDOR experiment, which are position 122, 362, 517, 528, 535, 554 and 603. Additional mutants, at position 295, 333 and 469 will also be investigated.

The interaction of both phytochromes with each other was also investigated by FRET, where Agp1 and Agp2 were labeled with Atto495 as donor and Atto565 as acceptor, respectively. FRET was measured after mixing both compounds. First results have indeed shown an interaction between both phytochromes. The impact of photoconversion on FRET will also be tested.

We investigated light-induced signal transduction by conjugation studies using a point mutation of Agp1 at the histidine kinase autophosphorylation site H528 and under different light conditions in order to find out how autophosphorylation is involved in signal transduction. The results showed a drastic reduction of the conjugation as compared to the wild type, suggesting that autophosphorylation is involved in conjugation. Furthermore, conjugation assays will be performed after knockout of the traA protein, which is supposed to be the first protein to induce the conjugation.

> OC118. Oral Communication
Symposium SENS-11 Short Communications on Photosensory Biology (Silvia Braslavsky)

EXPLORING THE FUNCTION OF THE IRON-SULFUR CLUSTER IN PROKARYOTIC (6-4) PHOTOLYASES

Authors: Gero Kaeser[1], Hongju Ma[1], Tilman Lamparter[1], Norbert Krauß[1]
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Photolyases are flavoproteins which are capable of repairing UV-damaged DNA via a light-triggered electron transfer to the lesion. Typical UV-damages are the cyclobutane pyrimidine dimers (CPD) and the (6-4) photoproducts which can result in a premature stop of replication or transcription and mutagenesis. These dimers are repaired by either CPD photolyases or (6-4) photolyases. The cryptochrome and photolyase family (CPF) consists of 7 classes, the CPD Classes I to III, the Cry-DASH proteins, the eukaryotic (6-4) photolyases and animal cryptochromes, the plant cryptochromes and the prokaryotic (6-4) photolyases (former FeS-BCP). Almost all photolyases and cryptochromes have an FAD cofactor. Additional antenna chromophores like MTHF, 8-HDF or DMRL, so far found in photolyases only, transfer energy of absorbed light to FAD which is then in its excited state for either DNA repair or photoreduction. For DNA-repair, after the recognition of the photoproduct, the lesion will be flipped into the binding pocket of the photolyase. After stimulation by light the closely located and fully reduced FAD transfers an electron to the lesion, which leads to the repair of the dimer.

With our recent discovery of PhrB, the founding member of the prokaryotic (6-4) photolyases, a third cofactor of photolyases belonging to this group, an iron-sulfur cluster has been identified (Oberpichler, Pierik et al. 2011). Although the role of the iron-sulfur cluster is not known, the structural fold around it resembles the fold of the large subunit from eukaryotic and archaeal primases (Zhang, Scheerer et al. 2013). In other proteins FeS-clusters have functions such as electron transfer (e.g. ferredoxin, photosystem I), in substrate binding and activation (sulphite reductase), in iron storage (polyferredoxin) and in protein structure (endonuclease III, MutY). Different mutants of PhrB lacking one or more of the highly conserved cysteins necessary for FeS-cluster binding were generated. However, all mutants were insoluble or not expressed (Graf, Wesslowski et al. 2015). By phylogenetic studies we found that members of a subgroup of prokaryotic 6-4 photolyases lack most or all these cysteine residues and have therefore no FeS cluster. We reasoned that comparative studies on a member of this group could clarify the role of the FeS cluster in PhrB. We therefore started with recombinant expression of a cyanobacterial homolog from *Prochlorococcus marinus*, termed PromaPL (accession number WP_011132061). This photolyase has only one of the conserved cysteins left (Ma, Holub et al. 2019).

We performed optimizations of expression and purification of PromaPL. With partially purified PromaPL we found that FAD is incorporated in ca. 10% of the protein. The protein repaired 6-4 lesion DNA in a Mg^{2+} dependent manner. In the future we plan to optimize recombinant expression by using a tac promoter to increase the expression level and an N-terminal GST-tag to generate more soluble protein and to establish a second step of affinity chromatography. Thereafter, further characterization of PromaPL and investigations on the effect of the iron-sulfur cluster are planned.

> OC119. Oral Communication

Symposium SENS-11 Short Communications on Photosensory Biology (Silvia Braslavsky)

LIGHTS ON AND ACTION – OPTOGENETIC CONTROL OF MICROBIAL CELL FACTORIES

Authors: Fabienne Hilgers[1], Nora Lisa Bitzenhofer[1], Yannic Sebastian Ackermann[1], Dennis Binder[1], Alina Burmeister[3,4], Alexander Grünberger[3,4], Anita Loeschcke[1], Fabian Hogenkamp[2], Jörg Pietruszka[2,3], Karl-Erich Jaeger[1,3], Thomas Drepper[1]
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Optimization of microbial production processes often requires precise control over targeted metabolic pathways and underlying regulatory networks. In the last decades, many different ways have been published to engineer and control such processes in bacteria. Only recently, optogenetic tools such as light-responsive switches have been implemented which, in contrast to conventional regulatory systems, enable a non-invasive control over cellular functions with unprecedented spatiotemporal resolution. Here, we report on the development and evaluation of different optogenetic *on* and *off* switches that allow for light-mediated control of gene expression or protein activity. In addition, we demonstrate the applicability of these switches for programming bacterial production processes with light.

Optogenetic *on* switches: Broadly applicable light-responsive expression systems were developed by employing photocaged inducer molecules including caged IPTG and arabinose [1,2]. Light-induced removal of the photosensitive protection group results in an intracellular release of inducer molecules and hence in an immediate induction of target gene expression. The biotechnological applicability of these phototriggers could be demonstrated e.g. for induction profiling in order to optimize the synthesis of heterologous proteins and secondary metabolites such as the anticancer compound violacein in *E. coli* [2,3].

Optogenetic *off* switches: Genetically encoded photosensitizers (PS) are proteins that produce reactive oxygen species upon illumination [4]. Due to this feature PS constitute suitable optogenetic tools for chromophore-assisted light inactivation (CALI) of target enzymes in living bacteria. By using the bifurcated biosynthetic pathway of the antibiotic tripyrrole prodigiosin, we could demonstrate gradual PS-mediated inhibition of PigC that catalyzes the final product-forming condensation reaction.

Because of their unique properties, these newly established optogenetic switches can be applied in the near future as versatile plug-and-play tools suitable for optimizing complex production processes in a broad range of different bacteria by light.

References

[1] Binder, D., Grünberger, A., Loeschcke, A., Probst, C., Bier, C., Pietruszka, J., Wiechert, W., Kohlheyer, D., Jaeger, K.-E., Drepper, T. **(2014)**. Light-responsive control of bacterial gene expression: Precise triggering of the lac promoter activity using photocaged IPTG. *Integrative Biology, 6(6), 755–765.*
[2] Binder, D., Bier, C., Grünberger, A., Drobietz, D., Hage-Hülsmann, J., Wandrey, G., Büchs, J., Kohlheyer, D., Loeschcke, A., Wiechert, W., Jaeger, K.-E., Pietruszka, J., Drepper, T. (**2016**). *ChemBioChem, 17(4), 296–299.*
[3] Wandrey, G., Bier, C., Binder, D., Hoffmann, K., Jaeger, K.-E., Pietruszka, J., Drepper, T. Büchs, J. **(2016)**. Light-induced gene expression with photocaged IPTG for induction profiling in a high-throughput screening system. *Microbial Cell Factories, 15(1), 63.*
[4] Endres, S., Wingen, M., Torra, J., Ruiz-González, R., Polen, T., Bosio, G., Bitzenhofer, N.L., Hilgers, F., Gensch, T., Nonell, S., Jaeger, K.-E., Drepper, T. **(2018)**. An optogenetic toolbox of LOV-based photosensitizers for light-driven killing of bacteria. *Scientific Reports, 8(1), 15021*.

> OC120. Oral Communication

Symposium SENS-11 Short Communications on Photosensory Biology (Silvia Braslavsky)

ENGINEERING OF SIMPLE ULTRASENSITIVE SWITCHES WITH OPTOGENETIC TOOLS

Authors: Jonathan Trauth[2], Sebastian Hepp[2], Sophia Hasenjäger[1], Juri Goenrich[1], Roberta Spadaccini[3], Lars-Oliver Essen[2], Christof Taxis[1]
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Optogenetic control of protein activity is a versatile technique to gain control over cellular processes for biomedical or biotechnology approaches due to the superior characteristics of light as regulatory signal. However, many optogenetic tools used for synthetic regulation of cellular events display a certain activity in darkness that might limit their application. The combination of two or more optogenetic tools that act in a synergistic way results in ultrasensitive switches with increased performance. We applied this concept successfully on optogenetic tools to regulate protein abundance as well as cAMP production using *Saccharomyces cerevisiae* as model organism. Regulation of protein abundance was achieved by combining a light-sensitive transcription factor with a light-activated degradation signal, which results in fast and nearly complete removal of the target upon illumination of the yeast cells. Similarly, we combined a photoactivatable adenylyl cyclase (PAC) with a degradation sequence that is active in darkness, resulting in stabilization and activation of the PAC upon light exposure of the yeast cells. In both cases, we observed increased switching ratios as well as fast-acting kinetics by combining appropriate optogenetic modules, which increases the number of possible applications. Overall, the modularity of optogenetic tools facilitated the combinatorial process and the concept should be transferable to other tools to achieve superior regulation of protein activity by light.

SYMPOSIUM COMMUNICATIONS

PHYSICAL & CHEMICAL PHOTOBIOLOGY

> IL312. Invited Lecture
Symposium PCHEM-1 Photopharmacology (Amadeu Llebaria)

MODERN OPTICAL APPROACHES FOR DISSECTING NEUROMODULATORY CIRCUITS AND SIGNALING IN BEHAVIOR

Authors: Michael Bruchas[PhD]
Presenting Author: Michael Bruchas
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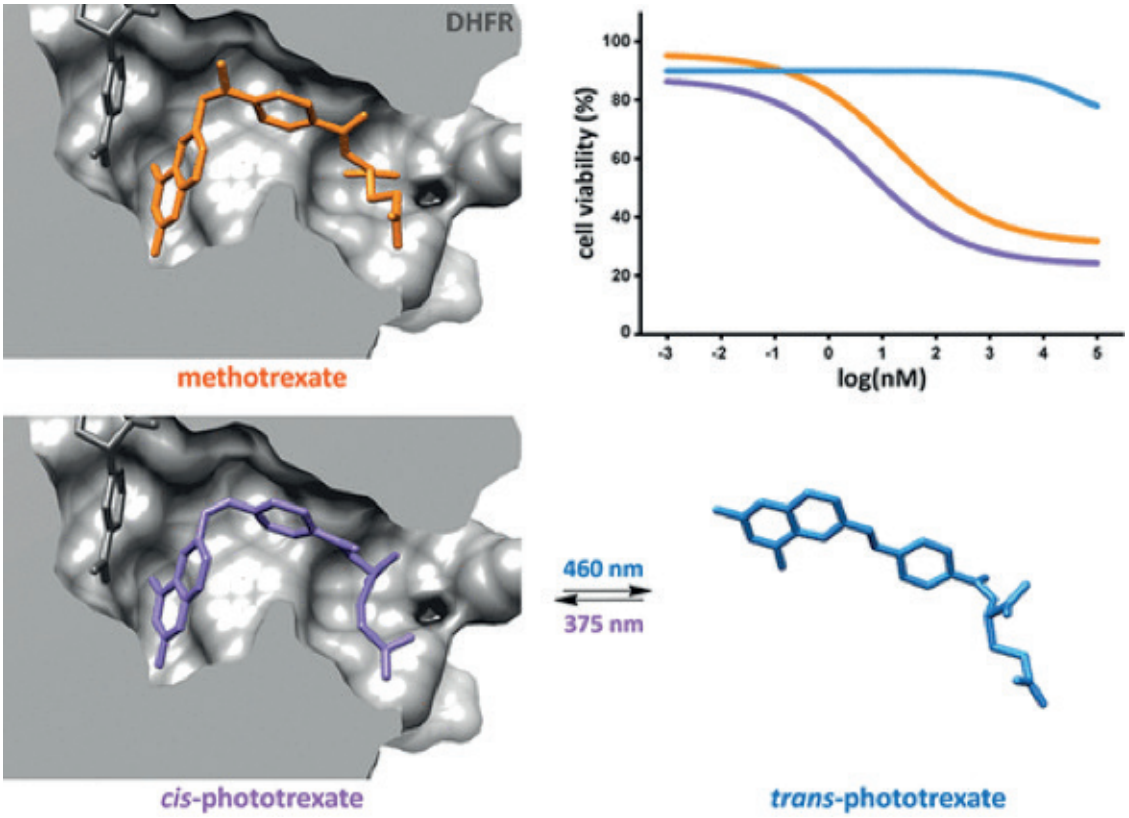
Stress and affective behaviors are largely controlled by specific neurotransmitters and their receptors in the central nervous system. Many of these signals are conveyed through activation of both neuropeptide (i.e. CRF and Opioid) and monoamine (norepinephrine, dopamine, serotonin) receptor systems. These receptors are seven transmembrane spanning G-protein coupled receptors (GPCR) and they can stimulate a variety of signaling cascades following neurotransmitter/neuropeptide release. The Bruchas laboratory uses a multimodal effort to uncover GPCR-mediated neuromodulation from the receptor, signaling, circuits, and systems level analysis. Here I will describe two recent technological developments in the laboratory. Specifically, this will include biological optical technology and hardware development for dissecting neuromodulation in vivo. I will first discuss recent advances in optogenetic technology including development of novel opto-GPCRs for interrogation of GPCR signaling in vivo, with spatiotemporal precision. I will also discuss advances in the engineering and implementation of wireless optofluidic devices for in vivo behavioral measures. This will include recent efforts by our group and collaborators in combining local pharmacology in discrete brain regions, with optogenetic control. In addition, I will share unpublished results using wireless drug delivery, and photo-pharmacology methods in vivo. In sum, I will highlight some recent optical approaches using both biological and engineering technology development from our laboratory that can be used to dissect the role of neuromodulation in motivated behavior.

Disclosure: Dr. Bruchas is a co-founder and scientific advisor for Neurolux, Inc.



> IL313. Invited Lecture
Symposium PCHEM-1 Photopharmacology (Amadeu Llebaria)

PHOTOCHROMIC ANTIFOLATE FOR LIGHT-ACTIVATED CANCER CHEMOTHERAPY

Authors: Carlo Matera[1,2], Alexandre Gomila-Juaneda[1,2], Núria Camarero[1,2], Michela Libergoli[1], Concepció Soler[3], Pau Gorostiza[1,2,4]
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Although chemotherapy is one of the most common treatments for cancer, its efficacy and tolerability are in many cases dramatically limited by off-target toxicity. A promising approach to improve cancer therapies is to activate the drugs exclusively at their desired place of action. In fact, in cancer types and stages that would benefit from a highly localized treatment, a precise spatiotemporal control over the activity of a cytotoxic agent would allow reducing the concentration of active compound outside the targeted region, improving the tolerability of the treatment. Light is a powerful tool in this respect: it offers unparalleled opportunities as a non-invasive regulatory signal for pharmacological applications because it can be delivered with high precision regarding space, time, intensity and wavelength. Alternative pharmacological strategies relying on the use of light have emerged during the last decades, but their intrinsic dependence on molecular oxygen and/or the irreversibility of activation of the cytotoxic species constitute major drawbacks. Photopharmacology allows a novel approach to address these problems, as it relies on the use of reversibly photoswitching target-specific drugs. We report here on the development and characterization of phototrexate, the first light-regulated inhibitor of the human DHFR, as a photochromic analog of methotrexate. Quantification of enzymatic activity, cell proliferation, and in vivo effects in zebrafish show that phototrexate behaves as a potent antifolate in its photoactivated *cis* configuration, and that it is nearly inactive in its dark-relaxed *trans* form. Thus, phototrexate constitutes a proof-of-concept to design light-regulated cytotoxic drugs and a step forward to develop targeted anticancer photochemotherapies.

> IL314. Invited Lecture
Symposium PCHEM-1 Photopharmacology (Amadeu Llebaria)

TOWARDS THERANOSTIC APPLICATIONS OF PHOTOPHARMACOLOGY

Authors: Wiktor Szymanski[1,2], Mark Hoorens[1,2], Friederike Reessing[1,2], Mickel Hansen[2], Jana Volaric[2], Ben Feringa[2]
Presenting Author: Wiktor Szymanski
1) Medical Imaging Center, University of Groningen, University Medical Center Groningen 2) Stratingh Institute for Chemistry, Faculty of Science and Engineering, University of Groningen

Light offers unparalled advantages in regulation of compound bioactivity (**photopharmacology**)[1-3] and as an input/output signal in **medical** (mostly optical) **imaging**.[4-5] Combination of those two paradigms along the principles of theranostics ("treat what you see, see what you treat") reuires light-responsive tools that, preferably in combination, enable both therapy and imaging.

I will present our efforts towards the discovery of such tools, focusing on new (i) photopharmacological agents, (ii) molecular photoresponsive tools and (iii) new light-responsive, MRI-active liposomal drug delivery agents.

Acknowledgements
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References

1. Lerch, M. M.; Hansen, M. J.; van Dam, G. M.; Szymanski, W.; Feringa, B. L. *Angew. Chem., Int. Ed.* **2016**, *55*, 10978−10999.
2. Broichhagen, J.; Frank, J. A.; Trauner, D. *Acc. Chem. Res.* **2015**, *48*, 1947−1960.
3. Hoorens, M.W.H.; Szymanski, W. Trends. Biochem. Sci. **2018**, 43, 567-575.
4. Weissleder R.; Ntziachristos V. *Nat. Med.* **2003**, *9*, 123-128.
5. Reeßing, F.; Szymanski, W. *Curr. Opin. Biotechnol.* **2019**, *58*, 9-18.

> IL315. Invited Lecture
Symposium PCHEM-1 Photopharmacology (Amadeu Llebaria)

PHOTOPHARMACOLOGY OF G PROTEIN-COUPLED ADENOSINE RECEPTORS

Authors: Francisco Ciruela[1,2], Kenneth A. Jacobson[3]
Presenting Author: Francisco Ciruela
1) Universitat de Barcelona-IIDBELL 2) Institut de Neurociències-Universitat de Barcelona 3) National Institutes of Health

Adenosine, a ubiquitous extracellular signaling molecule, acts through cell surface G protein-coupled receptors. These receptors control many physiological functions, thus becoming promising therapeutic targets in a wide range of pathological conditions. Yet, the ubiquity of adenosine receptors and the eventual lack of selectivity of adenosine-based drugs often reduced the therapeutic expectations. Photopharmacology is a novel approach based on the use of photosensitive drugs allowing spatiotemporal control of receptor function in a light-dependent manner, thus circumventing some of the classical pharmacology limitations. Accordingly, we developed light-sensitive drugs to photocontrol adenosine receptor's function both *in vitro* and *in vivo*. To this end, two type of adenosine-based photosensitive drugs were developed: i) Photswitchable; and ii) Photocaged.

MRS5543 is a photoisomerizable nucleoside derivative containing an aryl diazo linkage on the N(6) substituent Interestingly, while in dark conditions (*i.e.* relaxed isomer) it behaves as a full adenosine A_3 receptor (A_3R) and partial adenosine A_{2A} receptor ($A_{2A}R$) agonist, upon photoisomerization with blue light it turns into an $A_{2A}R$ antagonist[1]. Thus, MRS5543 is a photoswitchable purinergic drug that allow a light-dependent control of $A_{2A}R$ intrinsic activity. Conversely, MRS7145 is a photocaged $A_{2A}R$ antagonist which binds and block $A_{2A}R$ in a light-dependent manner both in cells and *in vivo*. Thus, precise fibreoptic brain irradiation allows MRS7145 uncaging and striatal $A_{2A}R$ blockade, thus fine-tuning $A_{2A}R$-dependent spontaneous locomotor activity and reversing pharmacologically-induced Parkinsonian-like behaviour[2].

Overall, the design and synthesis of light-operated adenosine receptor ligands opens new opportunities to widen the phototherapeutic window of adenosine receptors

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References

1. Bahamonde MI, Taura J, Paoletta S, Gakh AA, Chakraborty S, Hernando J *et al.* Photomodulation of G protein-coupled adenosine receptors by a novel light-switchable ligand. *Bioconjugate Chemistry* 2014; **25**: 1847–1854.
2. Taura J, Nolen EG, Cabré G, Hernando J, Squarcialupi L, López-Cano M *et al.* Remote control of movement disorders using a photoactive adenosine A 2A receptor antagonist. *Journal of Controlled Release* 2018; **283**: 135–142.

> IL316. Invited Lecture
Symposium PCHEM-1 Photopharmacology (Amadeu Llebaria)

ALLOSTERIC PHOTOSWITCHABLE LIGANDS TO CONTROL GPCR ACTIVITY WITH LIGHT

Authors: Amadeu Llebaria[MCS,]
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The administration of a photoactivated ligand in combination with illumination that is patterned in space and time can provide a novel degree of control and regulation of receptor activity. This method would allow precisely focusing the action of the ligand controlling the location and the temporal extension of its effects. When applied in vivo, the use of photopharmacology can reduce side effects by targeting receptors located in focused tissues, with potential for establishing personalized drug schedules to patient needs.

We have developed light-regulated negative allosteric modulators for metabotropic glutamate receptors (mGluRs). These include Alloswitch-1 and related phenylazopyridines [1] with NAM activity in mGlu5 and OptoGluNAM4.1, the first mGlu4 NAM active in vivo [2]. These photopharmacological tools are based in photoswitchable azobenzene scaffolds that show a robust activity dependent of the illumination conditions in cell assays. allowing real-time regulation of the intracellular effects of these GPCRs.

Moreover, when the molecules are applied in vivo and combined with external or internal light sources, we can register light dependent behavioral effects in zebra fish embryos, tadpoles and rodents, including some pain models. Thus, localized (in)activation with light of a specific area in the amygdala of live mice results in a regulation of chronic pain. [3] The key experiments involve a mGlu4 photoswitchable azobenzene ligand to control activity of endogenous receptors in vivo with light. With this molecule, we rapidly and reversibly inhibited chronic pain behavioral symptoms after illumination in the amygdala of rodent brain while measuring the painful response in the periphery. We have demonstrated a photopharmacological dynamic regulation of sensory and emotional information, bypassing central sensitization processes established for long periods of time and the validation of targeting local mGlu4 for persistent pain. This approach is effective to study the pharmacology of mGluRs and shows potential for spatiotemporal regulation of drugs targeting mGluRs.

References

[1] Gómez-Santacana, X. et al *ACS Cent. Sci*. **2017**, *3*, 81.
[2] Rovira X, et al *Cell Chem Biol*. **2016**, *23*, 929.
[3] Zussy C, et al. *Mol Psychiatry* **2018**, *23*, 509.

> IL317. Invited Lecture
Symposium PCHEM-1 Photopharmacology (Amadeu Llebaria)

RELIEVING PAIN THROUGH DEEP BRAIN PHOTOPHARMACOLOGICAL STIMULATION

Authors: Cyril Goudet[1]
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Chronic pain is among the most debilitating and costly afflictions in Europe. Unfortunately, the current treatment are not adequate, relieving pain in less than half of the patients and often leading to serious adverse side-effects. This emphasizes the crucial need for understanding the mechanisms regulating chronic pain to develop new analgesics.

Glutamate is the main neurotransmitter involved in the transmission of pain throughout the pain neuraxis. Dysregulation of glutamatergic transmission is involved in the development of central sensitization of the pain pathway underlying the sensory and anxio-depressive symptoms observed in patients with chronic pain.

We used photopharmacology to study glutamate-associated regulatory mechanisms involved in chronic pain. We specifically target amygdala, a key region of the brain linking pain sensation with negative emotions. Using newly designed freely diffusible photoswitchable allosteric modulator, we took control of a subtype of glutamate receptor (the mGlu4 receptor) by light in the brain of freely moving animals.

We demonstrated that sensory and anxio-depressive symptoms of chronic pain can be rapidly and reversibly alleviated though optical control of amygdala mGlu4 receptors. These findings could help to define novel and more precise therapeutic interventions for chronic pain, and exemplify the potential of *in vivo* photopharmacology.

> OC121. Oral Communication

Symposium PCHEM-1 Photopharmacology (Amadeu Llebaria)

SYNTHETIC PHOTOSWITCHABLE NEUROTRANSMITTERS BASED ON BRIDGED AZOBENZENES

Authors: Gisela Cabré[1], Aida Garrido-Charles[2], Àngels González-Lafont[1], Widukind Moormann[3], Daniel Langbehn[3], David Egea[1], Josep M. Lluch[1], Rainer Herges[3], Ramon Alibés[1], Félix Busqué[1], Pau Gorostiza[2,4,5], Jordi Hernando[1]
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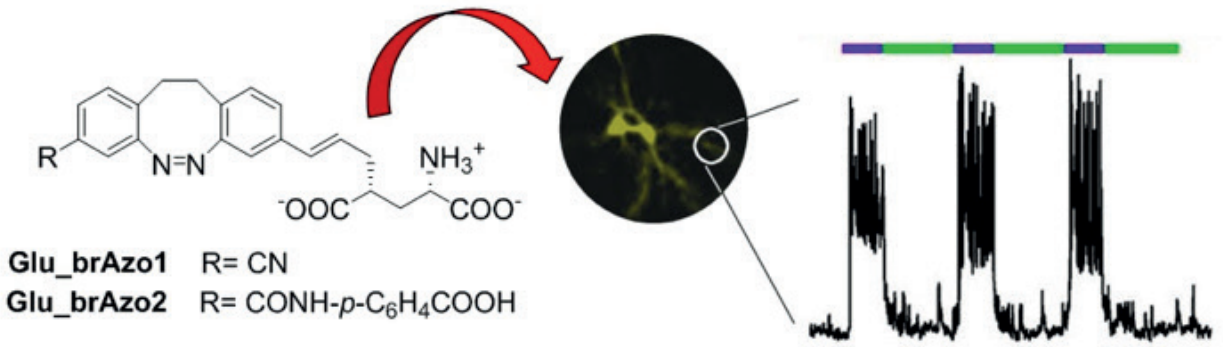
1) Universitat Autònoma de Barcelona 2) Institut de Bioenginyeria de Catalunya, Barcelona Institute of Science and Technology 3) Christian Albrechts University Kiel 4) Institució Catalana de Recerca i Estudis Avançats 5) Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina

Azoaromatic compounds are at the core of most photopharmacological tools developed to remotely control neuronal signaling (and other biological systems) with light.[1] This is the case of photochromic ligands (PCLs), light-activable drugs that are mainly prepared by tethering a pharmacologically-active unit to an azo photochrome.[1,2] *Trans-cis* photoisomerization of this group alters the interaction of the ligand with the target receptor, thus allowing photomodulation of its native response. Because of steric effects, azo-based PCLs are mainly active in their more thermodynamically stable, extended *trans* state, and only become inactive upon illumination and photoisomerization to their bent *cis* isomer.[1,2] Unfortunately, this severely hampers their use, since the opposite behavior would be preferred for most applications; i.e. PCLs should remain in their inactive state when administered in the dark, and be selectively photoactivated on demand under irradiation with both spatial and temporal precision.

To accomplish this objective while preserving the main design principles behind azo-based PCLs, we explored in this work the use of bridged azobenzenes, since they (a) should also favor *trans*-active behavior by photoswitching between extended *trans* and folded *cis* configurations, but (b) show *cis* thermal stability when bearing a short C2 bridge. In particular, we applied this strategy to the development of *trans*-active, *cis*-stable agonists of GluK1 and GluK2, two of the principal ionotropic glutamate receptors mediating excitatory neurotransmission in the central nervous systems.[3] With this aim, new photochromic ligands **Glu_brAzo1** and **Glu_brAzo2** were synthesized and tested on cultured cells and neurons. For the best of these compounds, selective neuronal firing upon irradiation without background activity in the dark could be achieved, thus largely improving the behavior of previously reported glutamate PCLs based on regular, *trans*-stable azobenzenes.

References
[1] Hüll, K.; Morstein, J.; Trauner, D. *Chem. Rev.* **2018**, 118, 10710-10747.
[2] Volgraf, M.; Gorostiza, P.; Szobota, S.; Helix, M. R.; Isacoff, E. Y.; Trauner, D. *J. Am. Chem. Soc.* **2007**, 129, 260-261.
[3] Traynelis, S. F.; Wollmuth, L. P.; McBain, C. J.; Menniti, F. S.; Vance, K. M.; Ogden, K. K.; Hansen, K. B.; Yuan, H.; Myers, S. J.; Dingledine, R. *Pharmacol. Rev.* **2010**, 62, 405–496.



> OC122. Oral Communication
Symposium PCHEM-1 Photopharmacology (Amadeu Llebaria)

EXPLORING THE EFFECTS OF PHOTOSWITCHING AGENTS ON KINASE ACTIVITY REGULATION

Authors: Theo Rodat[1], Dorian Schmidt[1], Linda Heintze[1], Mark W.H. Hoorens[2], Wiktor Szymanski[2], Lara Bußmann[3], Malte Kriegs[3], Christian Peifer[1]
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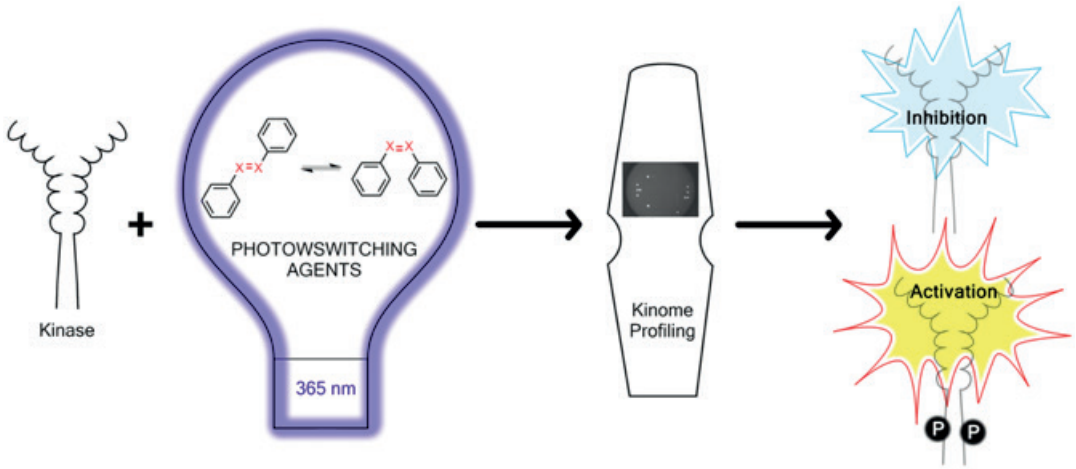
Protein kinases play important roles in virtually all cellular processes such as cell growth and differentiation. Given such a central function in cells, dysregulation of kinases can lead to cancer e.g. based on critical *gain-of-function* mutations. Consequently, protein kinases became an important drug target class in selective cancer chemotherapy.[1] During the development of protein kinase inhibitors from 2001 until today, many of the nowadays almost 50 approved kinase inhibitors are showing suboptimal specificity causing side effects. Moreover, similar to the situation with antibiotics, during therapy some cancers are developing resistances against kinase inhibitors.

An interesting approach to enhance selectivity and to increase local active agent concentration could consist in implementing a photoswitch into kinase inhibitors.[2] Triggered by irradiation, photoswitchable moieties change the configuration of a molecule. Thus, a photoswitchable protein kinase inhibitor could be switched “on” or “off” relative to its bioactive configuration by using a specific wavelength.

In this study, we describe photoswitchable kinase inhibitor design and biological evaluation on kinase specificity and efficacy in cellular assays by using the PamGene technology. Based on the approved protein kinase inhibitor axitinib, we report on a 43-fold difference in kinase activity from trans to cis.[3] In a second project, the kinase activity of $BRAF_{V600E}$ could be switched by factor 10.[4] However, the reported photoswitchable BRAF compound paradoxically also activates some kinases. Methodically, the PamGene approach can be used to assess photoswitchable kinase activity under controlled light conditions.

References

1. R. Roskoski, *Pharmacological Research* **2016**, *103*, 26.
2. K. Hüll, J. Morstein, D. Trauner, *Chemical Reviews* **2018**, *118*, 10710.
3. D. Schmidt, T. Rodat et al., *ChemMedChem* **2018**, *13*, 2415, doi: 10.1002/cmdc.201800531.
4. M. W. H. Hoorens, M. E. Ourailidou, T. Rodat et al., submitted at *European Journal of Medicinal Chemistry*



> OC123. Oral Communication
Symposium PCHEM-1 Photopharmacology (Amadeu Llebaria)

PHOTOACTIVATION OF METAL-BASED ANTICANCER PRODRUGS IN A BIOORTHOGONAL FASHION

Authors: Alessio Terenzi[1], Luca Salassa[1]
Presenting Author: Alessio Terenzi
1) Donostia International Physics Center

Metals play a key role in medicine with platinum drugs remaining among the most used chemotherapeutic agents in the clinics, either alone or within combination strategies. Transition metal complexes present a unique rich photochemistry and they have been intensively investigated as alternative agents for photochemotherapy. Various metal-based prodrug candidates have given promising results in vitro and vivo [1] with a Ru polypyridyl photosensitizer recently entering clinical trials for photodynamic therapy (PDT) in Canada [2]. Besides singlet-oxygen production, metal complexes can exert anticancer activity through different modes of action. In this contribution, we report on novel photoactivation strategies to control the biological action of metal-based prodrugs. In particular, we will describe how we make use of biorthogonal catalysis approaches to achieve outstanding efficiency and selectivity in the activation of metal-based anticancer agents and to shift their excitation energies to more convenient wavelengths [3-5]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement Phorau No 746976. The Spanish Ministry of Economy and Competitiveness is acknowledged for the grants CTQ2016-80844-R and BES-2013-065642.

References

[1] Alonso-de Castro S., Ruggiero E., Habtemariam A. and Salassa L., "Luminescent and Photoactive Transition Metal Complexes as Biomolecular Probes and Cellular Reagents", 1st edition, Springer, (2014).
[2] Mari C., Pierroz V., Ferrari S. and Gasser G., "Combination of Ru(II) complexes and light: new frontiers in cancer therapy", Chem. Sci., 6, (2015), pp 2660-2686.
[3] Alonso-de Castro S., Ruggiero E., Ruiz-de-Angulo A., Rezabal E., Mareque-Rivas J. C., Lopez X., López-Gallego F. and Salassa L., "Riboflavin as a bioorthogonal photocatalyst for the activation of a PtIV prodrug", Chem. Sci., 8, (2017), pp 4619-4625.
[4] Alonso-de Castro S., Cortajarena A. L., López-Gallego F. and Salassa L., "Bioorthogonal catalytic activation of platinum and ruthenium anticancer complexes by FAD and flavoproteins", Angew. Chem. Int. Ed., 57, (2018), pp 3143-3147.
[5] Alonso-de Castro S., Terenzi A., Hager S., Englinger B., Faraone A., Martínez J.C., Galanski M., Keppler B.K., Berger W., Salassa L., "Biological activity of PtIV prodrugs triggered by riboflavin-mediated bioorthogonal photocatalysis", Sci. Rep., 8, (2018), pp 17198

> P144. Poster

Symposium PCHEM-1 Photopharmacology (Amadeu Llebaria)

TRANEXAMIC ACID AMELIORATES NON-MELANOMA SKIN CANCER INDUCED BY LONG-TERM ULTRAVIOLET A IRRADIATION IN MICE

Authors: Keiichi Hiramoto[1], Yurika Yamate[1], Daijiro Sugiyama[2], Kazunari Matsuda[2], Yasutaka Iizuka[2], Tomohiko Yamaguchi[2]
Presenting Author: Keiichi Hiramoto
1) Department of Pharmaceutical Sciences, Suzuka University of Medical Science. 2) R&D Department, Daiichi Sankyo Healthcare Co., LTD.

Introduction

To date, there have been no treatments developed to ameliorate non-melanoma skin cancer induced by long-term exposure to ultraviolet A (UVA) irradiation (1). In this study, we examined the effects of tranexamic acid on long-term UVA-induced skin cancer.

Methods

We exposed the dorsal skin of male hairless mice to UVA at a dose of 110 kJ/m^2 using a FL20SBLB-A lamp three times weekly for 15 weeks after application of 7,12-dimethylbenz [a] anthracene (DMBA). During the experimental period, the mice were administered tranexamic acid (750 mg/kg/day) three times weekly.

Results and Discussion

We found that cancer development was ameliorated by administration of tranexamic acid. Furthermore, tranexamic acid treatment was observed to suppress increases in the plasma levels of matrix metalloproteinase-9 and interleukin (IL)-6, and skin expression of plasmin, C-C chemokine2, macrophages, signal transducer and activator of transcription (STAT)3, cyclin D, and vascular endothelial growth factor (VEGF)-A that occurred in mice subjected to long-term UVA irradiation.

Conclusions

These results indicated that the non-melanoma skin cancer induced by DMBA+UVA long-term irradiation is ameliorated by tranexamic acid through regulation of the plasmin/macrophage/IL-6/STAT3/cyclin D signal transmission pathway. In addition, this ameliorative effect against skin cancer may be mediated via inhibition of the IL-6-induced expression of VEGF-A (2).

Acknowledgement

This study was supported by JSPS KAKENHI.

Conflicts of interest

There are no conflicts of interest to declare.

References

(1) Hiramoto K et al., J. Clin. Exp. Dermatol. Res. 2018; 9: 5.
(2) Hiramoto K et al., Photochem. Photobiol. 2019; inpress.

> P145. Poster

Symposium PCHEM-1 Photopharmacology (Amadeu Llebaria)

PHOTOSWITCHABLE HYDROPHOBIC HELICAL PEPTIDE SHOWS SLOW AND MULTIEXPONENTIAL FOLDING KINETICS IN POPC MEMBRANES

Authors: Mónica Gutiérrez-Salazar[1], Eduardo Santamaría[2], Diego Sampedro[2], Víctor Lórenz-Fonfría[1]
Presenting Author: Mónica Gutiérrez-Salazar
1) Instituto de Ciencia Molecular, Universidad de Valencia 2) Centro de Investigación en Síntesis Química, Universidad de la Rioja

While the folding/unfolding kinetics of helical peptides in solution has been studied in detail, the folding/unfolding kinetics of helical hydrophobic peptides in lipidic membranes has received much less attention, largely because conventional experimental procedures to induce folding/unfolding in peptides are not suitable when working with lipidic membranes. An alternative approach to study folding/unfolding is the use of a photoswitch crosslinked to a peptide (Fig. 1). The photoswitch, an organic molecule that isomerizes between cis and trans conformations when irradiated with light of specific wavelengths, can induce reversible changes in the structure of the peptide, as shown previously for soluble peptides (1).

Here, we studied for the first time to our knowledge the folding/unfolding of a hydrophobic helical peptide in lipidic membranes with a photoswitch. A synthetic peptide from the KALP family, a model for transmembrane helices with alanines and leucines flanked by two lysines residues, was covalently crosslinked through two cysteine residues to an azobenzene photoswitch. The photoswitchable peptide, hereafter KCALP-azo, was characterized by UV-Vis and FTIR spectroscopies and shown to retain normal photo-isomerization and a highly helical structure in POPC membranes.

Time resolved studies by FTIR spectroscopy showed that the unfolding process of KCALP-azo was faster than our time-resolution of 100ms (Fig. 2). However, the folding process was extremely slow (minutes) and multiexponential (Fig. 2). This is in stark contrast with previous studies using photoswitchable helical soluble peptides, with folding/unfolding events completed in few microseconds (2). Interestingly, the folding of KCALP-azo was notably accelerated when using SDS micelles instead of POPC membranes. Overall, our results indicate that the folding of helical structures in a lipidic membrane is much more constrained than in detergent micelles or in solution, with multiple free energy barriers, as deduced by the much slower and complex folding kinetics in POPC membranes.

Acknowledgements

This work was supported by Ministerio de Economía, Industria y Competitividad (grant BFU2016-76805-P) and predoctoral contract BES-2017-080385. We thank Joachim Heberle for access to FTIR equipment.

References

1. Flint DG, Kumita JR, Smart OS, Woolley GA. Chem. Biol. 2002, 9, 391–397.
2. Bredenbeck J, Helbing J, Kumita JR, Woolley GA, Hamm P. Proc Natl Acad Sci U S A. 2005, 102(7), 2379-84.

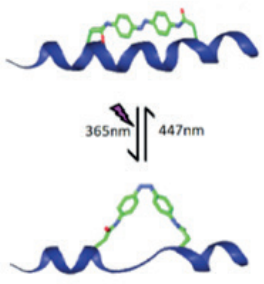


Figure 1. Molecular model of peptide cross-linked to a modified azobenzene. Upon irradiation, azobenzene switches between cis and trans isomers, modifying the secondary structure of the peptide.

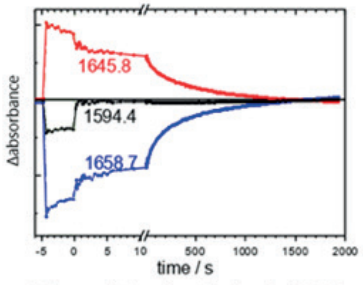


Figure 2. Time evolution of marker bands of KCALP-azo for azobenzene isomerization (black) and peptide conformational changes (blue and red), following UV (370 nm) illumination at -5s and blue (450 nm) illumination at 0s. The isomerization of the azobenzene and peptide unfolding is instantaneous, but peptide folding shows a complex and slow kinetics.

> P146. Poster

Symposium PCHEM-1 Photopharmacology (Amadeu Llebaria)

STUDIES OF FLUOROQUINOLONES AS ALKYLATING BOMBS

Authors: Cristina Anaya-Gonzalez[1], Inmaculada Andreu[2,3], Francisco Bosca[1]
Presenting Author: Cristina Anaya-Gonzalez
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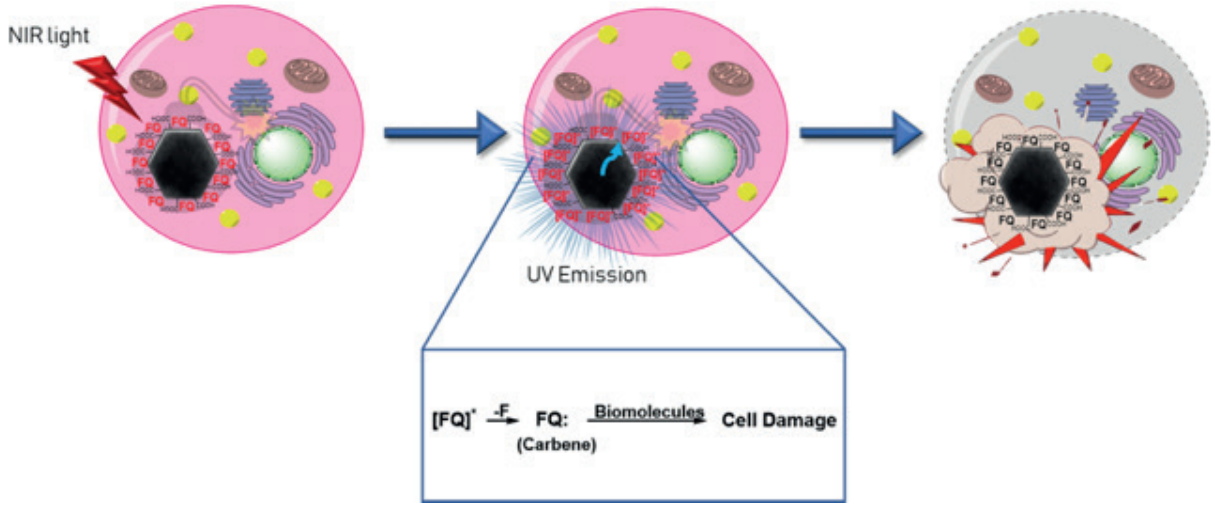
Fluoroquinolones (FQs) are modified quinolones with antibiotic and antineoplasic properties that inhibit Topoisomerase II.[1] In the last few years, antitumor activity that reduce all-cause mortality among cancer patients have been shown. [2] A recent study reported an enhancement of the FQ genotoxicity in eukaryotic systems by UV irradiation [3], which also confers to these drugs a potential property as photochemical therapeutic agent. Consequently, a large number of studies concerning the photophysical and photochemical properties of 6,8-dihalogenated FQs with and without the presence of biomolecules have been carried out.[4-7]

An unusual photodehalogenation of these FQs by heterolysis of their C8-halogen bond is a key point in the photoinduced biological damages.[7] In fact, two pathways have been proposed to explain the photoinduced adverse effects observed for the FQ. New FQs with enhanced photochemotherapeutic properties have been synthesised for improving the efficiency of the intramolecular photoreactions between FQ and biomolecules. In this context, upconversion nanoparticles (UCNPs), which are able to emit from UV-visible to near-infrared (NIR) light under NIR excitation[8], were functionalized with the new FQs. Photochemical and photophysical studies have been combined with *in vivo* cell culture experiments to determine the alkylating properties of the UNCPs-FQs complexes.

References

[1] C.E. Perrone, K.C.Takahashi, G.M.Williams, *Toxicol.Sci.* 69, 16–22 (2002).
[2] Paul, M.; Gafter-Gvili, A.; Fraser, A.; Leibovici, L., The anti-cancer effects of quinolone antibiotics? *Eur. J. Clin. Microbiol. Infect. Dis.*, *26* (11), 825-31; 2007.
[3] Perrone, C. E.; Takahashi, K. C.; Williams, G. M., Inhibition of human topoisomerase IIalpha by fluoroquinolones and ultraviolet A irradiation. *Toxicol. Sci.*, 69 (1), 16-22; 2002.
[4] Soldevila, S.; Bosca, F., Photoreactivity of Fluoroquinolones: Nature of Aryl Cations Generated in Water. *Org. Lett.*, 14 (15), 3940-3943; 2012.
[5] Albini, A.; Monti, S., Photophysics and photochemistry of fluoroquinolones. *Chem. Soc. Rev.*, 32 (4), 238-50; 2003.
[6] Fasani, E.; Manet, I.; Capobianco, M. L.; Monti, S.; Pretali, L.; Albini, A., Fluoroquinolones as potential photochemotherapeutic agents: covalent addition to guanosine monophosphate. *Org. Biomol. Chem.*, 8 (16), 3621-3; 2010.
[7] Soldevila, S.; Consuelo Cuquerella, M.; Lhiaubet-Vallet, V.; Edge, R.; Bosca, F., Seeking the mechanism responsible for fluoroquinolone photomutagenicity: a pulse radiolysis, steady-state, and laser flash photolysis study. *Free Radic. Biol. Med.*, 67, 417-25; 2014.
[8] Larsen, M. T.; Kuhlmann, M.; Hvam, M. L.; Howard, K. A. *Mol Cell Ther* 2016, 4, 3.



> P147. Poster

Symposium PCHEM-1 Photopharmacology (Amadeu Llebaria)

PHOTOCONTROL OF Z-DOMAINS WITH AZOBENZENE PHOTOSWITCHES

Authors: Nobuo Yasuike[1, 2], Jeffrey Youn[1], G. Andrew Woolley[1]
Presenting Author: Nobuo Yasuike
1) Department of Chemistry, University of Toronto, 80 St. George St., Toronto, M5S 3H6, Canada 2) JSR Corporation, 1-9-2, Higashi-Shinbashi, Minato-ku, Tokyo, 105-8640, Japan

Modifying the scaffolds of protein affinity reagents using azobenzene photoswitches is a generalizable, modular approach for obtaining light controllable bioactive agents. We applied this approach to the affibody scaffold based on the Z-domain derived from staphylococcal protein A. The Z-domain is well known as an immunoglobulin G (IgG) binding domain composed of three alpha helices, of which helices 1 & 2 bind to the F_c portion of IgG. We hypothesize that the secondary structure changes of helix 3, which is not part of the binding surface, can result in reduced interaction between each helix and affect the ability to bind to IgG.[1] We made eleven Z-domain mutants in which an azobenzene-based optical switch (IAC)[2] was intramolecularly bridged to helix 3 and tested the structural change by UV-light irradiation and binding to IgG. Whereas wild type Z-domains did not change secondary structure upon photoswitching, mutants that have a weaker interaction between helices than wild type underwent light-induced changes in helix content as measured by circular dichroism (CD) spectroscopy. The relationship between the secondary structural change of the mutant and its IgG binding property will be reported.

References

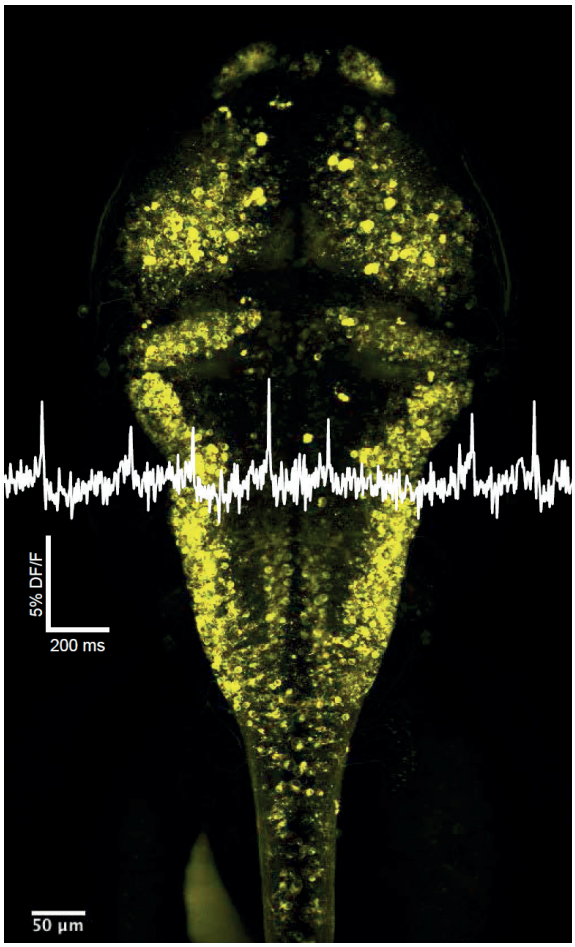
1. Andrew C. Braisted, James A. Wells, Proc. Natl. Acad. Sci. USA, 1996, 93 5688-5692
2. J. R. Kumita, O. S. Smart, G. A. Woolley, Proc. Natl. Acad. Sci. USA, 2000, 97, 3803-3808

> IL322. Invited Lecture
Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

FROM SINGLE-MOLECULE IMAGING TO THE BRAIN: A CIRCUITOUS ROUTE TO NEW NEURAL ACTIVITY INDICATORS

Authors: Luke Lavis[1]
Presenting Author: Luke Lavis
1) Janelia Research Campus, Howard Hughes Medical Institute

Small molecules remain important tools to probe or perturb biological systems. Designing chemical reagents for modern neuroscience remains a significant challenge, however, since the brain is the most complex and least accessible organ in the body. My lab initially focused on molecular tools for neuroscience, but these efforts largely failed. Frustrated by the brain, we instead began developing reagents for cell biology with the goal of creating bright, cell-permeable dyes for single-molecule imaging. Inspired by computational experiments, we discovered that replacing the *N*,*N*-dimethylamino substituents in the classic dye tetramethylrhodamine with four-membered azetidine rings greatly improved brightness and photostability. The novel substitution is generalizable to fluorophores from different structural classes and enables fine-tuning of the dyes' spectral and chemical properties. This effort yielded a palette of fluorophores useful in live-cell imaging experiments and we have since turned our focus back to the brain, learning that these dyes can also be delivered to neurons *in vivo*. This allows the construction of hybrid small-molecule:protein sensors with substantially higher brightness and photon yields, facilitating new functional imaging experiments to measure changes in voltage or [Ca^{2+}].



> IL318. Invited Lecture
Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

HARNESSING CYANINE REACTIVITY TO PREPARE NOVEL FLUOROPHORES FOR ADVANCED IMAGING APPLICATIONS

Authors: Martin Schnermann[1]
Presenting Author: Martin Schnermann
1) National Cancer Institute - IR Uncaging Chemistry: Discovery and Applications

Existing fluorescent probes derive from a small set of core scaffolds initially developed as laser dyes, and subsequently applied for biomedical research with minimal synthetic modification. Consequently, there exists a significant opportunity to develop molecules specifically tailored for use in modern imaging applications.Our efforts center on the discovery and application of novel long-wavelength cyanine fluorophores. To gain access to new molecules, we develop new synthetic transformations that modify the core chromophore unit. We have discovered a novel class of near-IR emitting heptamethine cyanines. These molecules contain a C4'-*O*-alkyl substituent that is installed through a *N*- to *O*-transposition reaction. The new fluorophores exhibit excellent labeling properties, with reduced covalent reactivity and improved *in vivo* tumor uptake compared to existing near-IR cyanines. We have also shown that conformationally restrained pentamethine cyanines can be accessed through a ring forming cascade. The resulting molecules exhibit improved fluorescence quantum yield (3- to 4-fold) and extended lifetime relative to typical pentamethine cyanines. Moreover, these fluorophores recover from hydride reduction with dramatically improved efficiency. These observations enable efficient single-molecule localization microscopy in oxygenated buffer without addition of thiols. Overall, these efforts involve a feedback loop between chemical studies focused on the design and synthesis of novel compounds and biological applications in advanced microscopy and *in vivo*imaging studies.

References

1. Nani, R. R.; Shaum, J. B.; Gorka, A. P.; **Schnermann, M. J.*** Electrophile-Integrating Smiles Rearrangement Provides Previously Inaccessible C4′-*O*-Alkyl Heptamethine Cyanine Fluorophores. *Org. Lett.* **2015**, *17*, 302.
2. Michie, M.M., Götz,R., Franke,C., Bowler,M., Kumari,M., Magidson, V., Levitus,M.; Loncarek, J., Sauer, M., **Schnermann, M. J.***Cyanine Conformational Restraint in the Far-Red Range. *J. Am. Chem. Soc.,***2017**, *139*, 12406.

> IL321. Invited Lecture
Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

FLUORESCENT MOLECULAR SWITCHES FOR SUPERRESOLUTION MICROSCOPIES

Authors: Mariano Bossi[1]
Presenting Author: Mariano Bossi
1) Max-Planck-Institute for Medical Research

Diarylethenes (DAEs) possess outstanding fatigue-resistant in organic solvents and polymer films. In particular, oxidized sulfones derivatives presenting an emissive closed isomer with up to 90% efficiency, allow for a reliable control and modulation of the fluorescence signal. However, their application in fluorescence bioimaging was always challenged by a poor performance and solubility in aqueous environments. In this presentation, I will introduce our lab efforts to produce switches that retain the fluorescence modulation in aqueous buffers at biologically relevant pH values, as free dyes and as bioconjugates. I will discuss the strategies to improve the solubility in water and the fatigue resistance (i.e. the number of switching cycles endured). I will also show different conjugation and targeting strategies to label biological structures of interest, as well as the application of the most promising markers in modern nanoscopy techniques, both based on stochastic (STORM) and targeted (RESOLFT) imaging approaches. In addition, I will present a series of adducts that can be switched only with visible light. Their principal advantage is to avoid the need of illumination with ultraviolet light, usually required by most DAEs, which is undesired in live-cell applications.

> IL319. Invited Lecture
Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

MAPPING MICROSCOPIC VISCOSITY AND TEMPERATURE USING MOLECULAR ROTORS

Authors: Marina Kuimova[1]
Presenting Author: Marina Kuimova
1) Chemistry Department, Imperial College London

Viscosity is one of the main factors which influence diffusion in condensed media. In a cell viscosity can play a role in several diffusion mediated processes, such as drug delivery, signalling and mass transport. Previously, alterations in viscosity in cells and organs have been linked to malfunction; however, mapping viscosity on a single-cell scale remains a challenge.

We have imaged viscosity inside lipid mono- and bi-layers, in cells and in atmospheric aerosol particles using fluorescent probes, called molecular rotors [1-2]. In molecular rotors the speed of rotation about a sterically hindered bond is viscosity-dependent, which strongly affects fluorescence lifetime or spectra of rotors, allowing fluorescence imaging. This approach enabled us to measure both the microscopic viscosity and temperature [2, 3] and monitor their temporal changes in real time. The talk will cover our recent developements of this technique, such as genetic and passive targeting or rotors [4, 5].

References

[1] M. K. Kuimova, *Phys Chem Chem Phys*, **2012**, 14, 12671
[2] A. Vyšniauskas et al, *Int. Rev. Phys. Chem.* **2018**, 37:2, 259
[3] A. Vyšniauskas, et al, *Chem. Sci.*, **2015**, 6, 5773
[4] J.E. Chambers et al, *ACS Nano*, **2018**, 12, 4398
[5] M. Kubankova et al, *Soft Matter*, **2018**, 14, 9466

> IL320. Invited Lecture
Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

FLUOROGENIC AND CHROMOGENIC DYES AND NANOPARTICLES AS BRIGHT PROBES FOR BIOLOGY

Authors: Andrey KlymchenkoUnive
Presenting Author: Andrey Klymchenko
1) Université de Strasbourg

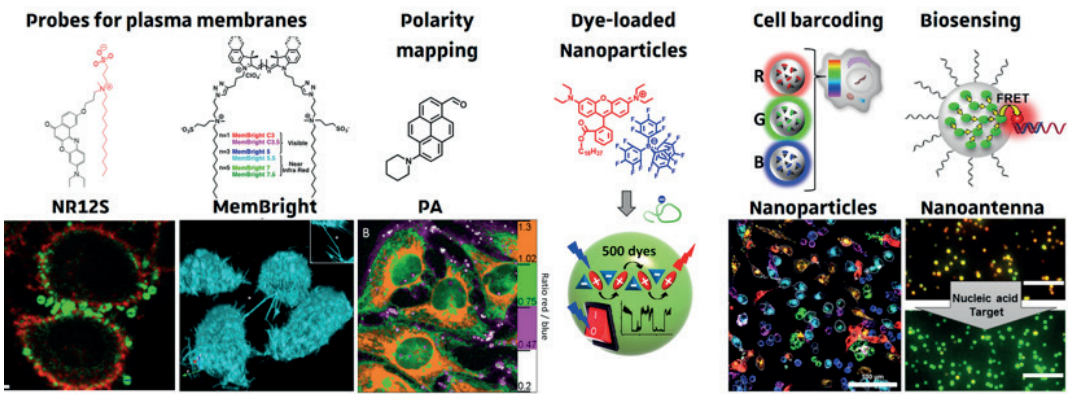
Fluorescent probes are essential tools for lighting up biomolecular processes and cellular structures. To implement fluorescence response by intensity (fluorogenic) or color (chromogenic), we exploited several mechanisms, such as solvatochromism and dye disassembly.[1] Based on solvatochromic dyes, we developed fluorescent membrane probes that change their color in response to apoptosis, lipid order in microdomains (rafts),[2] etc. To further meet the needs of advanced fluorescence microscopy, we recently introduced bright fluorogenic probes of different color for lipid droplets,[3] cell plasma membranes,[4] and intracellular lipid membranes.[5] Moreover, we introduced a series of probes for G protein coupled receptors that either change color or turn on fluorescence after binding to the target receptor in live cells.[6]

Brightness of dyes can be drastically increased by assembling them into nanoparticles, e.g. dye-loaded polymer nanoparticles (NPs).[7] First, to prevent dyes from self-quenching inside NPs, we used bulky hydrophobic counterions that act as spacers between dyes.[8] So far, we have already obtained polymer NPs with size ranging from 7 till 100 nm[9] and >100-fold higher brightness than quantum dots.[10] Moreover, using NPs of different color, we introduced a technique for long-term barcoding of living cells that allows tracking multiple cell populations in vitro and in vivo.[11] Second, the response of NPs to the target was achieved using an ultrafast communication of thousands of encapsulated dyes, that efficiently transfer energy to a single acceptor.[10] It enabled first detection of single dye molecules at ambient light excitation.[10] Based on these light-harvesting nanoantennas, we designed FRET-based color switching nanoprobes to detect target nucleic acids with single-molecule sensitivity.[12]

ERC consolidator grant BrightSens 648528 is acknowledged for the financial support.

References

(1) Klymchenko, A. S. *Acc. Chem. Res.* 2017, *50*, 366.
(2) Klymchenko, A. S.; Kreder, R. *Chem. Biol.* 2014, *21*, 97.
(3) Collot, et al *J. Am. Chem. Soc.* 2018, *140*, 5401.
(4) Collot, et al *Cell Chem. Biol.* 2018, doi: doi.org/10.1016/j.chembiol.2019.01.009
(5) Niko, Y.; Didier, P.; Mely, Y.; Konishi, G.; Klymchenko, A. S. *Sci. Rep.* 2016, *6*, 18870.
(6) Karpenko, et al, *J. Am. Chem. Soc.* 2015, *137*, 405.
(7) Reisch, A.; Klymchenko, A. S. *Small* 2016, *12*, 1968.
(8) Reisch, A.; Didier, P.; Richert, L.; Oncul, S.; Arntz, Y.; Mely, Y.; Klymchenko, A. S. *Nature Commun.* 2014, *5*, 4089.
(9) Reisch, A.; Heimburger, D.; Ernst, P.; Runser, A.; Didier, P.; Dujardin, D.; Klymchenko, A. S. *Adv. Funct. Mater.* 2018, *28,* 1805157.
(10) Trofymchuk, K.; Reisch, A.; Didier, P.; Fras, F.; Gilliot, P.; Mely, Y.; Klymchenko, A. S. *Nature Photonics* 2017, *11*, 657.
(11) Andreiuk, B.; Reisch, A.; Lindecker, M.; Follain, G.; Peyrieras, N.; Goetz, J. G.; Klymchenko, A. S. *Small* 2017, *13*.
(12) Melnychuk, N.; Klymchenko, A. S. *J. Am. Chem. Soc.* 2018, *140*, 10856.



> IL323. Invited Lecture
Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

LIVE CELL IMAGING OF LIPID PEROXIDATION AND ASSOCIATED BYPRODUCTS

Authors: Gonzalo Cosa[McGil]
Presenting Author: Gonzalo Cosa
1) McGill University/ Chemistry

Our ongoing interest on the role of lipid peroxidation and associated byproducts - including lipid derived electrophiles (LDEs) - in cellular physiology and pathology have led to developing a number of fluorogenic probes over the years intended to monitor lipid peroxyl radicals, electron transport in membranes, and how LDE react and evolve within cells. In this presentation I will describe the mechanism of action of the probes providing a rationale for the choices of trap and reporter (BODIPY dyes) segments on the basis of signal sensitivity (and the ensuing photo-physical -chemical processes), chemical selectivity and environment specificity [1]. I will provide recent examples for the use of the probes in bio-analytical assays and in state-of-the-art fluorescence imaging studies including super resolution imaging based on single molecule localization microscopy (SMLM) [1] of reactions in biological milieu [1e-f]. Imaging studies conducted on E. coli, HeLa, and primary neuronal culture cells will provide new insights on the role of reactive oxygen species (ROS) in the lipid membrane and cellular activity [1e-f].

Keywords
BODIPY dyes, fluorogenic probes, Reactive oxygen species, electrophiles, super resolution, Lipid membranes

References
[1] a) Greene, L. E.; Godin, R.; Cosa, G.; *J. Am. Chem. Soc.,* 2016, 138, 11327–11334. b) Belzile, M-. N.; Godin, R.; Durantini, A. M.; Cosa, G.; *J. Am. Chem. Soc.*, 2016, 138, 16388-16397. c) Lincoln, R.; Greene, L.; Bain, C.; Flores-Rizo, J. O.; Bohle, D. S.; Cosa, G.; *J. Phys. Chem. B,* 2015, 119, 2015, 4758-4765. d) Greene, L. E.; Lincoln, R.; Cosa, G.; *J. Am. Chem. Soc.,* 2017, 139, 15801-15811. e) Lincoln, R.; Greene, L. E.; Louisa, S.; Cosa, G.; *J. Am. Chem. Soc.*, 2017, 139, 16273-16281. f) Foret, M. K.; Do Carmo, S.; Lincoln, R.; Greene, L. E.; Zhang, W.; Cuello, A. C.; Cosa, G.; *Free Radic. Biol. Med.*, 2019, 130, 471-477.

> OC124. Oral Communication

Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

SMALL PARTICLES, BIG IMPROVEMENT: FLUORESCENT NANOPROBES FOR SINGLET OXYGEN AND OTHER REACTIVE OXYGEN SPECIES DETECTION IN BIOLOGICAL SYSTEMS

Authors: Roger Bresolí-Obach[1,2], Santi Nonell[1]
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Production of singlet oxygen ($^1O_2$) is a major component of anticancer and antimicrobial PDT.[1,2] Its facile generation by photosensitisation, together with its high reactivity against a wide variety of cellular and tissue components, make $^1O_2$ a highly attractive specie for oxidation-based treatment of localised diseases.[3] The amount of $^1O_2$ actually produced during PDT depends on several variables and is therefore difficult to control, which detracts from the efficacy and safety of PDT treatments.[4] Developing tools for monitoring $^1O_2$ during treatments can contribute to improve the clinical outcome. As part of our efforts towards this end, we report herewith the results in the development of fluorescent nanoprobes and their performance in cells.[5-7]

A number of nanoprobes for $^1O_2$ detection in biological systems have been developed, namely a polyacrylamide-based biocompatible fluorescent nanoprobe, the mesoporous silica-bound anthracene dipropionic acid and dichloro-dihydro-fluorescein diacetate. The reactivity against $^1O_2$ has been optimized by choosing appropriate linkers. The nanoparticle scaffolds shield the fluorescent probes from the external medium but not from $^1O_2$, thereby preventing unwanted interactions with proteins and the photosensitizer. Moreover, internalization by HeLa cancer cells or *E. coli* bacteria has been observed and intracellular $^1O_2$ and other ROS sensing has been demonstrated as well. The higher resistance to oxidation by air and to self-sensitized photooxidation, as well as lower affinity for interaction with proteins, make these nanoprobes safer and more reliable fluorescence markers for ROS in cells. The “nano” approach overcomes many of the shortcomings of molecular probes and is a useful strategy to extend their utility to complex biological systems.

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References

[1] Agostinis, P., *et al*. CA Cancer J. Clin., **61** (2011) 250-281.
[2] Wainwright, M., *et al.* Lancet Infect. Dis., **17** (2017) e49-e55.
[3] Nonell S., Flors. C. (editors). Singlet Oxygen. Applications in Biosciences and Nanosciences, The Royal Society of Chemistry, London, 2016.
[4] Pogue, B.W., *et al*. Phys. Med. Biol., **61** (2016) R57–R89.
[5] Bresolí-Obach, R., *et al.* Methods, **109** (2016) 64-72.
[6] Ruiz-González, R., Bresolí-Obach, R., *et al.* Angew. Chem. Int. Ed., **56** (2017) 2885-2888.
[7] Bresolí-Obach, R. *et al.* Photochem. Photobiol., **93** (2018) 1143-1150.

> OC125. Oral Communication
Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

ILLUMINATING BIOLOGICAL MICROENVIRONMENTS

Authors: Kylie Yang[1], Kathryn Leslie[1], Elizabeth New[1]
Presenting Author: Kylie Yang
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Introduction

Fluorescent probes now find widespread use as biological tools. They can be used to monitor live systems as dosing is minimally invasive, and they provide a way to visualise biological microenvironments such as redox state,[1] ion concentration,[2] hypoxia[3] and enzyme levels. Probes can be classified as either reversible or reaction based, and intensity based or ratiometric. Low concentrations of analytes can be monitored using reaction-based probes, as the fluorescence signal builds up over time and is amplified. Ratiometric probes provide better quantitative data as two wavelengths can be monitored to give a ratio independent of probe concentration.

In this work, we focus on detecting hypoxia, which is defined as a deficiency of oxygen in tissues.[3] Hypoxia is related to many health conditions including cancer, stroke and heart attack.

Methods

Fluorescent probes based on a bio-reductive sensing strategy were developed for 2D and 3D cell models, and visualised using live cell confocal fluorescence microscopy.

Results and Discussion

Perhaps one of the most striking differences between normal and cancerous tissue is the presence of tumour hypoxia.[4] Tumour spheroids are useful 3D models as they contain similar chemical gradients which give rise to proliferating, hypoxic and necrotic cells.[5] We successfully developed florescent probes for staining the hypoxic region with good contrast. Our probes are particularly suited for imaging live cells and tissue models.[3]

Conclusions

We developed a set of probes for monitoring hypoxia including some that are ratiometric and some that are intensity based. We applied them to study cancer cells, stem cells and spheroids.[3]

Acknowledgements

The authors acknowledge the support of an Australian Postgraduate Award, The University of Sydney Vice Chancellors Research Scholarship, The University of Sydney Nano Institute Postgraduate Supplementary Scholarship

Conflicts of Interest

None

References

1. Yang, K., Kolanowski, J. L. & New, E. J. Mitochondrially targeted fluorescent redox sensors. *Interface Focus* **7,** 20160105 (2017).
2. Kaur, A., Lim, Z., Yang, K. & New, E. J. Fluorescent Sensors for Biological Metal Ions. in *Comprehensive Supramolecular Chemistry II* 295–317 (Elsevier, 2017). doi:10.1016/B978-0-12-409547-2.12612-5
3. Yang, K. *et al.* Tailoring the properties of a hypoxia-responsive 1,8-naphthalimide for imaging applications. *Org. Biomol. Chem.* **16,** 619–624 (2018).
4. Hunter, F. W., Wouters, B. G. & Wilson, W. R. Hypoxia-activated prodrugs: paths forward in the era of personalised medicine. *Br. J. Cancer* **114,** 1071–1077 (2016).
5. Mehta, G., Hsiao, A. Y., Ingram, M., Luker, G. D. & Takayama, S. Opportunities and challenges for use of tumor spheroids as models to test drug delivery and efficacy. *J. Control. Release* **164,** 192–204 (2012).

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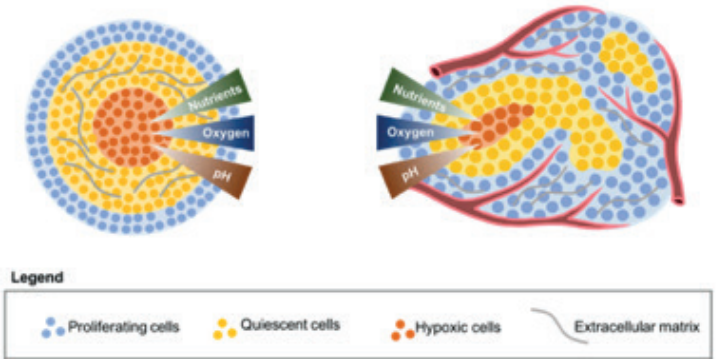
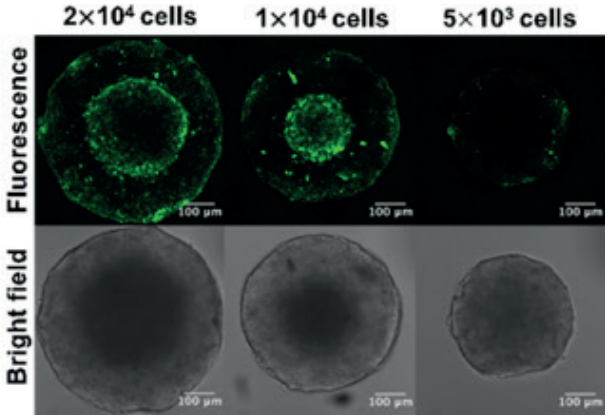

Nutrients
Oxygen
pH
Nutrients
Oxygen
pH
Legend
Proliferating cells
Quiescent cells
Hypoxic cells
Extracellular matrix


2×10⁴ cells
1×10⁴ cells
5×10³ cells
Fluorescence
Bright field
100 µm

> P148. Poster

Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

PROBING CELL SURFACE BIOTIN RECEPTORS WITH RATIONALLY DESIGNED FLUOROGENIC DIMERIC SQUARAINES

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Biotin is an essential vitamin playing its role in cellular carbohydrate, amino acid and lipid metabolism. Unlike bacteria, mammalian cell machinery does not produce biotin, therefore biotin is supplemented exogenously. There are 2 cellular transport systems for biotin: a high-affinity biotin transporter (≤ 10 nM) and sodium-dependent multivitamin transporter SMVT (≥ 10 μM). There is evidence that expression of biotin receptors (BRs) is correlated with cancer. While biotin receptors have been attractive target as the drug delivery system to tumor, there is lack of robust imaging probes for clinical diagnostic of BRs in cancerous cells and evaluation of new targeted therapeutics.[1,2]

Fluorogenic probes are particularly adapted for deciphering biological processes with background-free imaging. [3,4] Although environment-sensitive biotin probes have been developed for BRs imaging operating in visible region, detection of BRs at low concentration requires superior brightness. Moreover, there is a particular demand in development of probes operating in far-red and near-infrared (NIR) region to image deeper in the tissues and potentially *in vivo*. Squaraines are particularly interesting for probe development since they have exceptional molar extinction coefficient (~ 300.000 $M^{-1}cm^{-1}$) and their absorption and emission are in the far-red to NIR window. Recently, we showed that squaraine dimers can report the cellular membrane oxytocin receptor.[5] In this work, we rationally designed and synthesized 4 variants of biotinylated fluorogenic squaraines, evaluated their capacity and specificity to image BRs in a bright and specific manner. Rational design improved receptor-selectivity allowing the visualization of live cell surface BRs. We believe that our work will contribute in providing a guideline to rational design of environment-sensitive fluorogenic sensors.

References

(1) Ren, W. X.; Han, J.; Uhm, S.; Jang, Y. J.; Kang, C.; Kim, J.-H.; Kim, J. S. Recent Development of Biotin Conjugation in Biological Imaging, Sensing, and Target Delivery. *Chem. Commun.* **2015**, *51* (52), 10403–10418. https://doi.org/10.1039/C5CC03075G.
(2) Gao, M.; Yu, F.; Lv, C.; Choo, J.; Chen, L. Fluorescent Chemical Probes for Accurate Tumor Diagnosis and Targeting Therapy. *Chem. Soc. Rev.* **2017**, *46* (8), 2237–2271. https://doi.org/10.1039/C6CS00908E.
(3) Zhu, H.; Fan, J.; Du, J.; Peng, X. Fluorescent Probes for Sensing and Imaging within Specific Cellular Organelles. *Acc. Chem. Res.* **2016**, *49* (10), 2115–2126. https://doi.org/10.1021/acs.accounts.6b00292.
(4) Klymchenko, A. S. Solvatochromic and Fluorogenic Dyes as Environment-Sensitive Probes: Design and Biological Applications. *Acc. Chem. Res.* **2017**, *50* (2), 366–375. https://doi.org/10.1021/acs.accounts.6b00517.
(5) Karpenko, I. A.; Collot, M.; Richert, L.; Valencia, C.; Villa, P.; Mély, Y.; Hibert, M.; Bonnet, D.; Klymchenko, A. S. Fluorogenic Squaraine Dimers with Polarity-Sensitive Folding As Bright Far-Red Probes for Background-Free Bioimaging. *Journal of the American Chemical Society* **2015**, *137* (1), 405–412. https://doi.org/10.1021/ja5111267.

> P149. Poster

Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

LIGHT-HARVESTING POLYMERIC NANOPARTICLES FOR AMPLIFIED DETECTION OF NUCLEIC ACID CANCER MARKERS

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Presenting Author: Nina Melnychuk
1) Nanochemistry and Bioimaging group, Laboratoire de Bioimagerie et Pathologies, UMR 7021 CNRS, Faculté de Pharmacie, Université de Strasbourg

Direct sequence-specific detection of several copies of nucleic acids remains a challenge due to poor brightness of existing fluorescent probes. That is why it requires an amplification technique, such as polymerase chain reaction (PCR), which includes a complex mixture of expensive reagents, sophisticated equipment and well-trained staff. Our goal is to develop alternative amplification strategy to achieve simple one-step ultrasensitive detection of nucleic acids.

To this end, we developed ultrabright dye-loaded polymeric nanoparticles[1,2], which feature controlled small size and exceptional brightness because they encapsulate >1000 dyes per particle. We found that these nanoparticles operate as giant light-harvesting nanoantenna capable to amplify emission of single dye molecules >1000 fold, which enables for the first time detection of single molecules in sunlight excitation conditions.[3]

Here, based on these light-harvesting nanoantennas we developed the nanoprobe for nucleic acids, by covalent modification of the polymer nanoparticles with oligonucleotides. In such system single nucleic acid hybridization triggers fluorescence response, equivalent to hundreds of molecular probes. Therefore, the nanoprobe enables sequence-specific detection of target nucleic acids at a very low limit of detection (0.25 pM), which can be achieved only by molecular multiplication (like PCR).[4] We also show that the performance of our nanoprobe can be further improved, reaching sensitivity down to detection of single nucleic acid molecules. The developed nanoprobes constitute a new powerful platform for rapid and sensitive nucleic acid detection.

Acknowledgements
This work is supported by ERC consolidator grant BrightSens 648528.

References

1) Reisch, A.; Didier, P.; Richert, L.; Oncul, S.; Arntz, Y.; Mely, Y.; Klymchenko, A. S. Nature Communications 2014, 5, 4089.
2) Reisch, A.; Klymchenko, A.S. Small 2016, 12, 1968.
3) Trofymchuk, K.; Reisch, A.; Didier, P.; Fras, F.; Gilliot, P.; Mely, Y.; Klymchenko, A. S. Nature Photonics 2017, 11, 657.
4) Melnychuk N. & Klymchenko A., J. Am. Chem. Soc. 2018, 140, 10856.

> P150. Poster

Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

ISOMERIZATION VERSUS FLUORESCENCE: CASE STUDY OF VOLTAGE SENSORS QUASARS

Authors: Arita Silapetere[1], Yusaku Hontani[2], Patrick E. Konold[2], John T.M. Kennis[2], Peter Hegemann[1]
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QuasArs are recently reported archaerhodopsin-based voltage sensor [Hochbaum etal. 2014]. They posses a novel property for microbial rhodopsins – voltage dependent fluorescence, where more positive membrane potential values yield higher fluorescence. This property of the QuasArs is of a particular interest for neuronal imaging, allowing to track action potential propagation in neuronswith high spatiotemporal resolution. Knowledge of the photodynamics would enable rational design of new tools and increase of fluorescence quantum yield, which is of great demand for further voltage-sensing applications. However, until now the photochemical dynamics that underlay the functionality of the QuasArs are still unclear. We have performed femtoesecondspectroscopyto probeQuasAr1 and QuasAr2. This method allows to study the dynamics of the exited state, which is directly related to the fluorescence properties. It was observed thattheexcitedstate S_1decayswith two time constants: ~3ps and ~30ps, with only minor formation of isomerized photoproduct. With comparison of the excited-state dynamics of QuarAr1 and QuasAr2, we propose that the shorter lived excited state species is involved in retinal isomerization and the longer lived excited state species is the dominant one for fluorescence. The counterion in QuasAr1 and QuasAr2 is differently charged and is likely the origin of the difference of the isomerisation efficiency and fluorescence quantum yield.

> P151. Poster

Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

SOLVATOCHROMIC MEMBRANE PROBES FOR SUPER-RESOLUTION IMAGING OF LIPID ORDER

Authors: Dmytro I. Danylchuk[1], Seonah Moon[2], Ke Xu[2], Andrey S. Klymchenko[1]
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The complex heterogeneous nature of cell plasma membranes raised intensive research in the last two decades, stimulated by the hypothesis of coexistence of ordered and disordered lipid phases (lipid rafts).[1, 2] The challenge to visualize lipid rafts is linked to their nanoscopic size, dynamic nature and non-flat geometry of plasma membranes.[3] In the present work, we aimed to develop a methodology that allows us accessing with nanoscopic precision both morphology and lipid order of the plasma membrane of live cells. This was achieved through a tailor-made design of fluorescent membrane probes based on the solvatochromic Nile Red fluorophore, the emission color of which directly reflects the lipid order as it is directly linked to local polarity and hydration.[4] Two types of probes were developed. The first one is a high-affinity membrane probe for classical measurements of lipid order at cell plasma membranes, which is an improved analogue of previously developed NR12S,[5] featuring higher brightness and photostability combined with lower phototoxicity. The second one is a low-affinity membrane probe, exhibiting effective ON/OFF switching on cell membrane reversible binding, which is optimal for spectrally resolved PAINT super-resolution microscopy. In contrast to Nile Red, commonly used in PAINT,[6] the new probe stains exclusively the plasma membrane, while also showing improved performance in super-resolution imaging. This probe enabled us to show a connection between nanoscopic morphology of the cell surface and the lipid order. The developed methodology opens new opportunities in nanoscale cartography of lipid order of cell membranes.

References

1. Sezgin E, *et al.* The mystery of membrane organization: composition, regulation and roles of lipid rafts. *Nat. Rev. Mol. Cell Biol.* **18**, 361-374 (2017).
2. Lingwood D, Simons K. Lipid Rafts As a Membrane-Organizing Principle. *Science* **327**, 46-50 (2010).
3. Stone MB, *et al.* Super-Resolution Microscopy: Shedding Light on the Cellular Plasma Membrane. *Chem. Rev* **117**, 7457-7477 (2017).
4. Klymchenko A. S, Kreder R. Fluorescent probes for lipid rafts: from model membranes to living cells. *Chem Biol.* **21**, 97-113 (2014).
5. Kucherak, *et al* Switchable Nile Red-based probe for cholesterol and lipid order at the outer leaflet of biomembranes. *J. Am. Chem. Soc.* **132**, 4907–4916 (2010).
6. Yan R, *et al*, Spectrally Resolved and Functional Super-resolution Microscopy via Ultrahigh-Throughput Single-Molecule Spectroscopy. *Acc. Chem. Res.* **51**, 697-705 (2018).

> P152. Poster

Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

DESIGNING ULTRABRIGHT DYE-LOADED POLYMER NANOPARTICLES FOR INTRACELLULAR IMAGING

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Presenting Author: Anne Runser
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Dye-loaded polymer nanoparticles (NPs) have become powerful tools for fluorescence imaging.[1] Their exceptional brightness makes them promising tools for tracking single biomolecules inside cells. But what are the size requirements needed for intracellular imaging? In this work we assembled a series of fluorescent polymer NPs with different sizes to study this question. For this we synthetized methyl methacrylate copolymers containing different amounts of positive or negative charged groups such as carboxylate, sulfonate and ammonium. The introduction of a few charged groups per polymer chain can strongly reduce the diameter of particles prepared through nanoprecipitation.[2] Furthermore, we achieved a fine size modulation by adding salt in the aqueous phase during nanoprecipitation. With these different features, the diameter of polymer NPs could be tuned from 50 to 7 nm.[3] The encapsulation of a high amount of fluorescent cationic dyes associated to a bulky hydrophobic counterion in NPs make them tenfold brighter than quantum dots,[4] and allows their tracking at the single-particle level. In order to study their behavior in cells, these NPs were introduced in the cytoplasm through microinjection. Observing their spreading and diffusion showed that only NPs smaller than a critical size of about 23 nm reach easily the whole cytosol. These ultrasmall dye-loaded polymer NPs have a great potential for diverse applications including high-speed tracking of single biomolecules with high localization precision.

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References

[1] Reisch, Klymchenko Small 12, 15 (2016), 1968-1992.
[2] Reisch, Runser, Arntz, Mely, and Klymchenko ACS Nano 9, 5 (2015), 5104-5116.
[3] Reisch, Heimburger, Ernst, Runser, Didier, Dujardin, Klymchenko Adv. Funct. Mater. 28, 48 (2018).
[4] Reisch, et al. Nat. Commun. 5, (2014), 4089.

> P153. Poster

Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

IMAGING DYE-LOADED POLYMER NANOPARTICLES IN FIXED CELLS: THE ROLE OF PARTICLE SIZE

Authors: Sylvie Egloff[1], Anne Runser[1], Nina Melnychuk[1], Andreas Reisch[1], Andrey Klymchenko[1]
Presenting Author: Sylvie Egloff
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Fluorescent polymer nanoparticles (NPs) encapsulating large quantity of dyes, so-called dye-loaded polymer NPs have attracted growing interest in bioimaging over the past years thanks to their high brightness, biocompatibility, flexibility in terms of cargo loading [1]. Previously, our team developed a concept of encapsulation of cationic dyes with bulky counterions into polymer NPs, that allows obtaning ultrabright nanoparticles. Moreover, we showed that small size of polymer NPs (from 10 to 100 nm, depending on the polymer)[2,3] can be obtained through nanoprecipitation of specially designed charged polymers. More recently, we found that NPs below a critical size of 23 nm is a requirement for spreading throughout the cytosol of living cells [4]. However, to be used inside the cells for bioimaging, they should be first delivered into the cytosol. Here, we asked a question: what is the critical particle size to reach the cytosol of fixed cells? To answer it, we incubated PEG-coated fluorescent polymer NPs of varied size (10 to 40 nm) with fixed Hela cells and studied by fluorescent microscopy. It was found that the smallest NPs in the series can reach and move freely in the cytosol and in the nucleus, whereas the largest cannot reach the nucleus anymore, but they are still able to get into the cytosol. Subsequently, we performed similar experiments but using polymeric NPs functionalized with nucleic acids [5]. Remarkably, DNA-coated NPs of 60 nm diameter remained at the cell surface, while 28-nm NPs entered fixed cells and spread in the cytosol. In conclusion, small size of NPs (<30 nm) is required to reach the cytosol of fixed cells, which will allow diverse applications such as detection of single molecules inside cells.

References

1. Reisch, A. & Klymchenko, A. S. Fluorescent Polymer Nanoparticles Based on Dyes: Seeking Brighter Tools for Bioimaging. *Small* **12**, 1968–1992 (2016).
2. Reisch, A. *et al.* Collective fluorescence switching of counterion-assembled dyes in polymer nanoparticles. *Nat. Commun.* **5**, 4089 (2014).
3. Reisch, A., Runser, A., Arntz, Y., Mély, Y. & Klymchenko, A. S. Charge-controlled nanoprecipitation as a modular approach to ultrasmall polymer nanocarriers: making bright and stable nanoparticles. *ACS Nano* **9**, 5104–5116 (2015).
4. Reisch, A. *et al.* Protein-Sized Dye-Loaded Polymer Nanoparticles for Free Particle Diffusion in Cytosol. *Adv. Funct. Mater.* **28**, 1805157 (2018).
5. Melnychuk, N. & Klymchenko, A. S. DNA-Functionalized Dye-Loaded Polymeric Nanoparticles: Ultrabright FRET Platform for Amplified Detection of Nucleic Acids. *J. Am. Chem. Soc.* **140**, 10856–10865 (2018).

> P154. Poster

Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

Investigation of the Compounds Influencing the Melting Temperature of Oligonucleotide Probes

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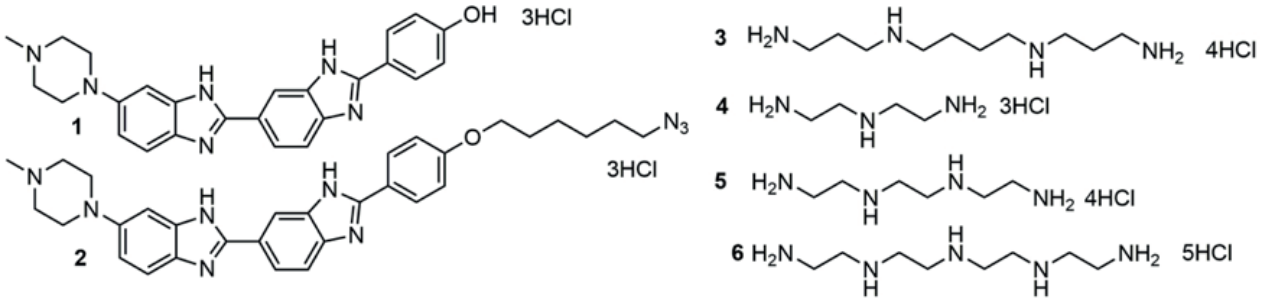
Real-time PCR is widely used method in various research fields. The use of longer probes is often not optimal for mismatch discrimination because of low melting temperature difference between a probe with complementary target and a probe with mismatched target. Shorter oligonucleotide probes can be advantageous in this case. On the other hand, low melting temperature of these shorter probes is major complication for practical use in real-time PCR. Minor groove binders (MGB) or intercalating dyes can stabilize the duplex and increase the melting temperature, e.g. biogenic polyamines have strong interaction with DNA and RNA.[1] In this work, several compounds were selected to test their capability to stabilize the duplex and increase melting temperature. Commonly known MGB Hoechst 33258 (**1**),[2] its modified derivative **2**, naturally occurring spermine **3**, three artificial polyamines (**4**-**6**) and several acridine derivatives were tested for their capability to increase melting temperature of shorter probes. Modified Hoechst 33258 (**2**) and several acridine derivatives were prepared in our laboratory.

The study was supported by Technology Agency of the Czech Republic (TH03010251) and The Grant Agency of Charles University (994218).

References

1. OUAMEUR, A. A.; TAJMIR-RIAHI, H. A.: J. Biol. Chem. 40, 2004, 279.
2. LUKINAVIČIUS, G.; BLAUKOPF, C.; PERSHAGEN, E.; SCHENA, A.; REYMOND, L.; DERIVERY, E.; JOHNSSON, K. Nat. Commun. 6, 2015, 8497.



> P155. Poster

Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

SOLVATOFLUOROCHROMISM OF CONJUGATED 4-METHOXYPHENYL-PYRIDINIUM ELECTRON DONOR-ACCEPTOR PAIRS AS NEW PROBES FOR MICROHETEROGENEOUS SYSTEMS

Authors: Carolina Aliaga[1, 2], Matías Vidal[1], Camila Pastenes[1,2], Marcos C. Rezende[1], Moisés Domínguez[1]
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1) Universidad de Santiago de Chile 2) Centro para el Desarrollo de la Nanociencia y la Nanotecnología

Pyridinium-based dyes have been used as chemical probes for gathering information about homogeneous[1]and microheterogeneous[2] environments. In this context, four new fluorescent dyes (**1-4**), composed of a 4-methoxyphenyl-pyridinium electron donor-acceptor pair, were synthesized and their solvatofluorochromism was investigated in thirteen different solvents with a wide range of polarities. Their different solvatofluorochromism behavior was rationalized by consideration of the relative regioisomeric conjugation of the donor and acceptor groups, the presence of a phenyl spacer between the donor and acceptor rings, and the increased rigidity and planarity of an analogous tetrahydrodibenzacridinium acceptor system. Their behaviour was further rationalized by theoretical calculations of the absorption and emission processes for the four dyes dissolved in dichloromethane.

Authors declare no Conflicts of Interest.

Acknowledgements
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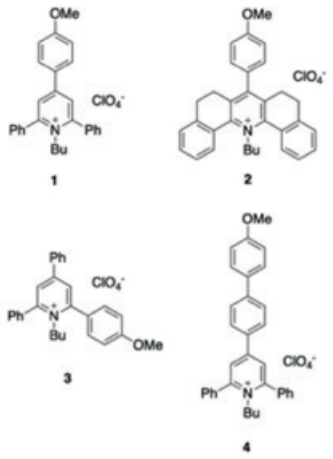
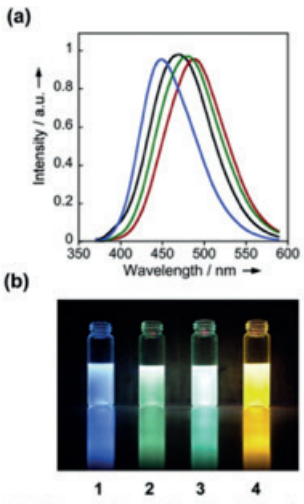
References

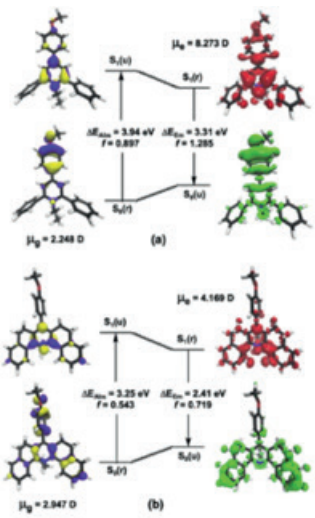
1. Machado V.G., Stock R.I., Reichardt C. Pyridinium N-phenolate betaine dyes. *Chem Rev.***2014**, *114*, 10429-10475.
2. Aliaga C., Rezende M.C., Tirapegui C. “A new probe for hydrogen abstraction and radical detection” *Tetrahedron***2009***65*, 6025-6028.



New 4-methoxyphenyl-N-butylpyridinium dyes **1–4** studied in this work.



(a) Fluorosolvatochromic behaviour of dye **1**: normalized emission spectra measured in N,N-dimethylformamide (red), ethanol (green), 1-octanol (black), and dichloromethane (blue); (b) fluorescent solutions of dyes **1–4** dissolved in dichloromethane ($c \approx 5 \times 10^{-5}$ M).



Calculated molecular structures for the HOMO S_0 and LUMO $S_1(u)$and electron-density difference distributions for the excited $S_1(r)$ and ground state $S_0(u)$ of dyes **1** (a) and **2** (b)

> P156. Poster

Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

SUPRAMOLECULAR INTERACTION OF TETRAPYRAZINOPORPHYRAZINES USED IN OLIGODEOXYNUCLEOTIDE PROBES

Authors: Jiri Demuth[1], Michal Kantor[1], Petr Zimcik[1], Veronika Novakova[1]
Presenting Author: Jiri Demuth
1) Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Kralove, Charles University, Czech Republic

Alkylamino substituted azaphthalocyanines (AzaPcs) have interesting spectral properties – they absorb in a wide range of UV-vis spectrum from 300 to 600 nm and quench fluorescence of other compounds. In non-coordinating solvents, these AzaPcs form J-dimers (Fig. 1) due to their planar aromatic core[1] that may affect their application as quenchers in oligodeoxynucleotide (ODN) probes.[2] The tendency to aggregation can be driven by peripheral substitution. The goal of this project was to compare influence of two different peripheral substituents on behavior of ODN probes. The preparation of quenchers was performed by standard procedures for synthesis of AzaPcs finished by unsymmetrical cycloteramerization. After the synthesis and purification, the ability of AzaPcs (alone and after binding to ODN probes) to form J-dimers was investigated. Addition of pyridine to solution cause disassembly of J-dimers, both AzaPcs alone or attached to ODN probe ODN probes were tested in a model of Taq-man assay (Fig. 2). In addition, the real time PCR tests were done to evaluate advantages and disadvantages of different substitution at quencher in real conditions.
The study was supported by Grant Agency of Charles University (1168217) and SVV 260 401.

References

1. NOVAKOVA, V.; ZIMCIK, P.; KOPECKY, K.; MILETIN, M.; KUNES, J.; LANG, K.: Eur. J. Org. Chem, 2008, (19), 3260-3263.
2. DEMUTH, J.; KUCERA, R., KOPECKY, K.; HAVLINOVA, Z.; LIBRA, A.; NOVAKOVA, V.; MILETIN, M.; ZIMCIK, P.: Chem. - Eur. J. 2018, 24 (38), 9658-9666.

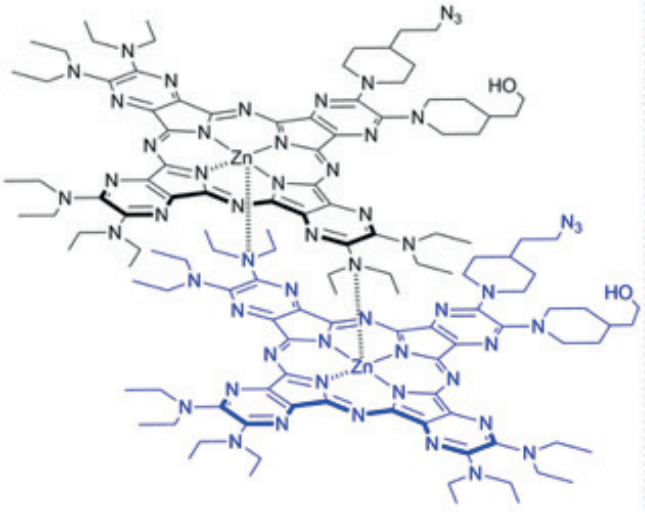


Fig. 1 - Example of AzaPc J-dimer

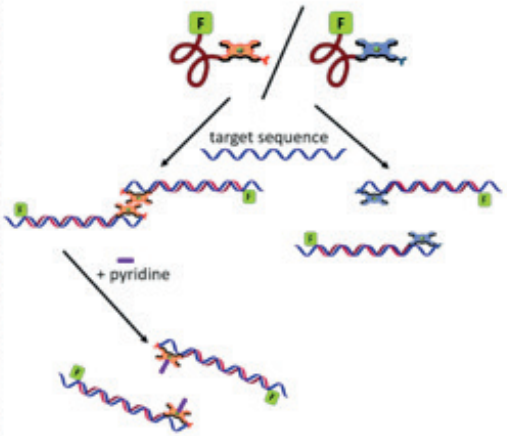


Fig. 2 - Scheme of behaviour of probes

> P157. Poster

Symposium PCHEM-2 Fluorescent probes (Gonzalo Cosa)

ENERGY TRANSFER BETWEEN PRODAN AND BORON-DIPYRROMETHEN (BODIPY) IN A MOLECULAR DYAD AND ON SURFACE OF SILICA NANOPARTICLES UPON ONE- AND TWO-PHOTON EXCITATION

Authors: Vladimir Stamenkovic[1], Daniel Collado[1,2], Ezequiel Perez Inestrosa[1,2]
Presenting Author: Vladimir Stamenkovic
1) Andalusian Centre for Nanomedicine and Biotechnology-BIONAND 2) Departamiento de Química Orgánica, Universidad de Málaga-IBIMA

Förster resonance energy transfer (FRET) is one of the major mechanisms widely used in biological research, due to its excellent characteristics such as a single excitation wavelength and a large pseudo-Stokes shift [1]. The aim of this study was to evaluate the efficiency of energy transfer between two different fluorophores -prodan and bodipy, in the form of a covalently linked molecular dyad, as well as randomly distributed on the surface of silica nanospheres, and characterize them under one and two photon excitations.

The molecular dyad was synthesized by forming an amide bridge between the two fluorophores. Previously prepared amino-functionalized silica nanoparticles were labelled with carboxy-functionalized prodan and bodipy molecules at 1:1 amino to carboxy group ratio, while the prodan:bodipy ratio was varied, ranging from 1:0 to 5:1. The photophisycal properties of the molecule and particles were then studied with a fluorimeter, as well as a confocal microscope for two-photon absorption (TPA). Additionally, the practical application of the dyad was tested in HeLa cells.

The dyad displays emission maximum at 533 nm with almost complete energy transfer. Two photons absorption spectra were obtained by comparison with a Rodamine B sample as reference, and it was shown that TPA comes from the prodan analog at 700 nm and shows a maximum of 108 GM. Dyad is easily internalized by the cells and no signs of morphological damage are observed. A closer inspection of the images revealed that the probe is preferentially localized in the cytoplasm and does not enter the cell nucleus. From the emission spectra of nanoparticle dispersions, it can be concluded that after direct excitation of the prodan molecule at 360 nm, the bodipy emission shows linear growth with increasing prodan content. Emission maximum is detected at 540 nm. Prodan peak is also detectable in the case of nanoparticles, indicating incomplete energy transfer, possibly because of distance and distribution of fluorophores on nanoparticle surface. TPA recordings show similar results, comparable to that of the dyad.

Based on the mentioned results, it can be said that the prodan-bodipy pair displays good fluorescence energy transfer pair that can be used in different platforms, including nanomaterials, with successful TPA properties. Further *in vivo* and *in vitro* applications should be explored, such as confocal microscopy probes and diagnostic tests.

Acknowledgements
The present study has been supported by Andalusian Regional Ministry Health (grants: PI-0250-2016); by the European Regional Development Fund (ERDF) and “Plan Propio Universidad de Málaga” (UMA-Andalucía-TECH). This project has received funding from the European Union\’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 713721.

References

[1] Shah S. et al., FRET study in oligopeptide-linked donor–acceptor system in PVA matrix. Methods Appl. Fluoresc. 2016, 4, 047002.

> IL326. Invited Lecture
Symposium PCHEM-3 Nanobioplasmonics (Belinda Heine)

A PHOTOTHERMAL LATERAL FLOW TEST FOR VISUAL POINT OF CARE DETECTION

Authors: Jesus M. de la Fuente[1]
Presenting Author: Jesus M. de la Fuente
1) Instituto de Ciencia de Materiales de Aragón, CSIC-Universidad de Zaragoza & CIBER-BBN, Spain.

The rapid development of cost-effective and efficient biosensors has had a profound worldwide socioeconomic impact. Advances in the fields of microelectronics, materials science and nanotechnology have been vital to the implementation of enhanced sensing platforms aimed at providing alternatives to traditional analytical methods. Further, current sensing platforms are often slow, unreliable and expensive; in particular, when ultralow concentration detection is required. Although progress in the biosensors field typically focuses on clinical point-of-care diagnosis, a clear demand exists in areas such as food safety regulation, environmental policy, military and the arts, where time, cost, portability and ease-of-use of the device are critical. The grand aim of our research is to develop an innovative, rapid, inexpensive, versatile and sensitive thermal transduction biosensor for the ultralow detection and quantification of relevant proteins such as tumoral markers. This novel sensing technology uses detection biomolecules linked to plasmonic gold nanoprisms which serve as thermal transducers by based on a lateral flow immunoassay and a “sandwich” recognition strategy with capture biomolecules immobilized on a dual-active nitrocellulose membrane support/thermosensitive paper that subsequently functions as photographic and tracing detection element. Although the ambitious thermal sensing device proposed here will be prototyped using simple analytes, this research goes significantly beyond the current state-of-the-art in nanoplasmonics and biosensing by proposing the development and elaboration of an almost universal paper-based thermal sensing device. This technology will be implemented and validated by applying it to a specific problem, gastrointestinal and prostate cancer diagnosis.

> IL325. Invited Lecture
Symposium PCHEM-3 Nanobioplasmonics (Belinda Heine)

PLASMONICS WITH VIRUSES

Authors: Amy S. Blum[1]
Presenting Author: Amy S. Blum
1) McGill University

Although there have been many advances in synthesizing nanoparticles, the assembly of these materials into deterministic and controllable patterns remains a major challenge. Biological systems operate at the nanoscale, building structural components with great chemical specificity that enable the processes of life. These successes result from billions of years of evolution. By adapting them to our needs, it is possible to utilize well-defined and well-controlled scaffolds to produce materials with novel properties that result from precise ordering on the nanoscale, such as a negative index of refraction, signal enhancement for spectroscopy, or evanescent wave focusing for superlenses. Utilizing viruses and viral proteins as templates is a relatively new idea that could have a large impact in nanotechnology and bioengineering. This approach offers the promise of exquisite control for positioning on the nanoscale, since the position of each coat protein within a virus-like particle is precisely defined, and self-assembly into homogenous micron-scale particles occurs spontaneously.

We use tobacco mosaic virus (TMV) coat protein as a template to self-assemble nanoparticles. This approach uses spatial arrangement instead of nanoparticle size, shape, or composition to control optical properties through the collective interactions between neighboring nanoparticles. Surface plasmons are resonant oscillations in the free electrons of a metal that are excited through interaction with light at the resonant wavelength. The effect of these plasmons is to focus incident light at the resonant wavelength into very small volumes near metal surfaces, leading to very intense local fields. In addition, these plasmonic oscillations can couple together, giving rise to more complex modes like plasmonic ring resonances that can be used to tune their response to incident light.

Here, we present robust covalent techniques using TMV coat protein as a template to produce nanostructured materials with novel properties. By exploiting the self-assembling properties and chemical addressability of TMV coat protein, we can utilize site-directed mutagenesis and bioconjugation strategies to produce highly symmetrical plasmonic nanorings, as evidenced by transmission electron microscopy (TEM). Theoretical models suggest that these rings may display an induced magnetic response at optical frequencies, and ensemble spectroscopic measurements reveal intriguing optical properties. Optical effects can be tuned by the introduction of a nanoparticle in the center of the rings through a pH dependent electrostatic interaction. Preliminary dark field scattering data, obtained for individual surface bound ring structures, is remarkably consistent with ensemble measurements, demonstrating that the observed optical properties arise from the ring structures. Thus, we show the utility of biotemplates in generating nanostructured building blocks for advanced materials.

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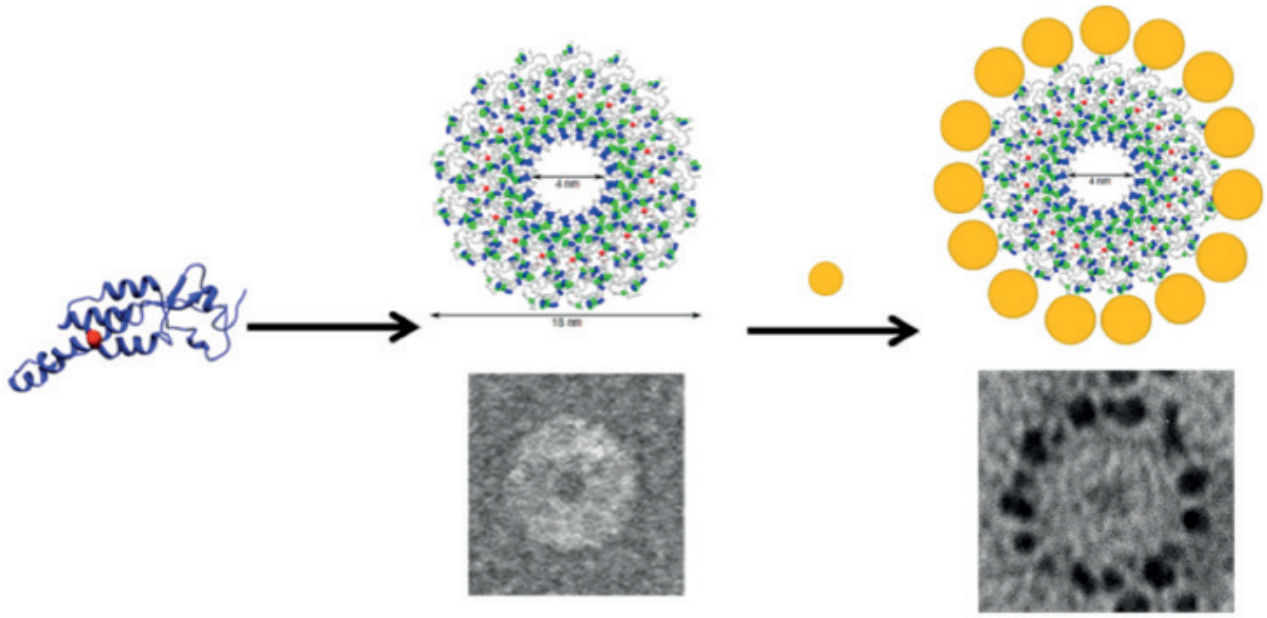
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> IL324. Invited Lecture
Symposium PCHEM-3 Nanobioplasmonics (Belinda Heine)

APPLICATION OF SURFACE-ENHANCED RAMAN SCATTERING (SERS) FOR IMAGING BACTERIAL BIOACTIVE METABOLITES

Authors: Gustavo Bodelón[1], Luis M. Liz-Marzán[1, 2], Jorge Pérez-Juste[1], Isabel Pastoriza-Santos[1]
Presenting Author: Gustavo Bodelon
1) Department of Chemistry-Physics and Biomedical Research Centre (CINBIO), Universidade de Vigo, 36310 Vigo, Spain 2) Bionanoplasmonics Laboratory, CIC biomaGUNE, Paseo de Miramón 182, 20014 Donostia-San Sebastián, Spain 3) Ikerbasque, Basque Foundation for Science, 48013 Bilbao, Spain 4) Biomedical Research Networking Center in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), 20014 Donostia-San Sebastián, Spain

In nature bacteria live in microbial communities shaped by the action of bioactive metabolites secreted by the residing microorganisms. The identification and tracking of such chemical exchange processes, such as Quorum Sensing, in live populations is fundamental for understanding their impact in microbial community and function. Herein we demonstrate the application of surface enhanced Raman scattering (SERS) as an imaging tool for the non-invasive detection and visualization of bioactive metabolites secreted by bacterial populations. The SERS-based approach not only provides a complementary tool to investigate the chemistry underpinning microbial communities, but it can also be implemented for the ultrasensitive detection and monitoring of microbial metabolites with potential pharmacological, or biotechnological interest.

> IL327. Invited Lecture
Symposium PCHEM-3 Nanobioplasmonics (Belinda Heine)

PHOTOLUMINESCENCE PROPERTIES OF BIOGENIC AND CHEMOGENIC SELENIUM NANOPARTICLES

Authors: Elena Piacenza[1], Alessandro Presentato[2], Silvia Lampis[2], Giovanni Vallini[2], Belinda Heyne[1], Raymond J. Turner[1]
Presenting Author: Elena Piacenza
1) University of Calgary 2) University of Verona

Introduction
The physical-chemical properties of selenium nanomaterials (SeNMs) empower their use to generate Se-based devices to apply in high-tech applications [1], or biomedicine [2-3]. However, SeNM optical and photoluminescence (PL) properties are scarcely investigated, representing an important gap to be filled. Here, PL features of biogenic Se nanoparticles (bioSeNPs) previously described [4] as compared to chemogenic ones (chSeNPs) [5] are investigated.

Methods
BioSeNPs were produced and recovered from *Stenotrophomonas maltophilia* SeITE02 as described elsewhere [4], while chSeNPs were synthesized according to [5]. Bio and chSeNPs were characterized by Transmission Electron Microscopy (Hitachi H7650-TEM) [6], and NP average diameter was evaluated measuring 100 randomly chosen NPs with ImageJ software. NP optical properties were investigated by UV/VIS/NIR DH-2000_BAL Mikropack and Nanolog/Fluorolog-3-169 2iHR320 spectrofluorimeter (4 nm slits), while Easylife LS (Photon Technology International) was used for lifetime fluorescence.

Results and Discussion
TEM imaging of bioSeNPs highlighted individual spheres of ca. 70 nm enclosed in an electron-dense material containing biomolecules [4] likely involved in NP thermodynamic stability , while chSeNPs were clustered together, however having average diameter similar to bioSeNPs. Ch and bioSeNPs showed a similar optical and PL properties, with absorption centered at 320 nm [5,6], and emission (λ_{em}) maxima at 450 nm upon excitation (λ_{exc}) at 400 nm, although λ_{exc} ranging from 300 to 400 [7] nm determined fluorescence emission. As λ_{exc} increased, a red-shift of λ_{em} maxima was measured, likely due to subpopulations of NPs having different sizes [8]. The contribution of different NPs to the PL was also confirmed by lifetime measurements, indicating multiple fluorescent species decaying between 2 and 20 nanoseconds.

Conclusions
This work strengthens the use of SeITE02 as *eco-friendly* catalyst to produce SeNPs with optical and PL properties comparable to chSeNPs. Thus, bioSeNPs can be applied to develop *green* SeNM tools for new application avenues.

Conflicts of interest
The authors declare no conflicts of interest.

References
1 Piacenza E, Presentato A, Zonaro E, Lampis S, Vallini G, Turner RJ *Phys Sci Rev*. 2018, 3, 20170100
2 Piacenza E, Presentato A, Zonaro E, Lemire JA, Demeter M, Vallini G, Turner RJ, Lampis S. *Microb Biotechnol.* 2017, 10, 804-818
3 Ahmad MS, Yasser MM, Sholkamy EN, Ali AM, Mehanni MM. *Int J Nanomed.* 2015, 10, 3389-3401
4 Piacenza E, Presentato A, Ambrosi E, Speghini A, Turner RJ, Vallini G, Lampis S. *Front Microbiol.* 2018, 9, 3178
5 Li Q, Chen T, Yang F, Liu J, Zheng W. *Mater Lett.* 2010, 64, 614-617
6 Presentato A, Piacenza E, Anikovskiy M, Cappelletti M, Zannoni D, Turner RJ. *Microb Cell Fact.* 2016, 15, 208
7 Khalid A, Tran PA, Norello R, Simpson DA, O'Connor AJ, Tomljenovic-Hanic S. *Nanoscale.* 2016, 8, 557 3376-3385
8 Stengl V, Henych J. *Nanoscale*. 2013, 5, 3387-3394

> IL328. Invited Lecture
Symposium PCHEM-3 Nanobioplasmonics (Belinda Heine)

PLASMON-ENHANCED SINGLET OXYGEN PRODUCTION USING HYBRID METALLIC NANOPARTICLES

Authors: Belinda Heyne[1], Nicolas Macia[1], Vladimir Kabanov[1], Mélanie Côté-Cyr[1], Santi Nonell[2], Roger Bresoli-Obach[2]
Presenting Author: Belinda Heyne
1) Chemistry Department, University of Calgary, 2500 University Drive NW, Calgary, AB, Canada, T2N 1N4 2) Institut Quimic de Sarria, Universitat Ramon Llull, Barcelona 08029, Spain

As one of the most versatile reactive oxygen species and a well-known cytotoxic agent, singlet oxygen, the electronically excited form of molecular oxygen, is at the forefront of a vast window of applications, such as photodynamic inactivation of micro-organisms, an alternative strategy to traditional antimicrobial drugs. In recent years, growing attention has emerged on plasmonic nanoparticles as promising candidates for improved photodynamic inactivation. These nanoparticles are known to interact with nearby photosensitizer molecules via a plasmon-enhanced phenomenon which can significantly alter their singlet oxygen production. Factors affecting the extent of this interaction are numerous and include the photosensitizer-metal separation distance, the degree of spectral overlap between the surface plasmon band and the photosensitizer absorption profile, and the metal nanoparticle core size and composition. In the first part of this presentation, the next generation of metal-enhanced singlet oxygen nanoplatforms exploiting the lightning rod effect, or plasmon hot spots, in anisotropic (non-spherical) metal nanoparticles will be described. We report the synthesis of Rose Bengal (RB) decorated silica-coated silver nanocubes (Ag@SiO_2-RB) with silica shell thicknesses ranging from 5 to 50 nm based on an optimized protocol yielding highly homogeneous Ag nanocubes. Using two different singlet oxygen detection techniques, the Ag@SiO_2-RB nanocubes were benchmarked for singlet oxygen production against the Ag@SiO_2-RB nanospheres previously reported by our group. Our study revealed that there is a dependence upon the metal core morphology for optimum metal-enhanced singlet oxygen production, for which the nanocubes were shown to produce more singlet oxygen compared to the nanospheres. Additionally, the Ag@SiO_2-RB nanocubes also showed improved antimicrobial activities in photodynamic inactivation experiments using both gram-positive and -negative bacteria model strains. In the second part of this presentation, investigation of the mechanism of plasmon-enhanced singlet oxygen production will be discussed. Mechanisms insights were obtained by synthesizing RB decorated silica-coated silver, gold and silver:gold alloy nanoparticles. Experimental and computational (finite-domain time-difference) results corroborate to suggest that plasmon-enhanced singlet oxygen production results from an interplay of near- and far-field interactions between the plasmonic nanomaterials and the photosensitizer molecules.

> OC126. Oral Communication
Symposium PCHEM-3 Nanobioplasmonics (Belinda Heine)

EVALUATION OF CELL DAMAGE INDUCED BY IRRADIATED ZINC-PHTHALOCYANINE-GOLD DENDRIMERIC NANOPARTICLES IN A BREAST CANCER CELL LINE

Authors: Heidi Abrahamse[1]
Presenting Author: Heidi Abrahamse
1) Laser Research Centre, University of Johannesburg

Introduction

Cancer is a non-communicable disease that occurs following a mutation in the genes which control cell growth. Breast cancer is the most diagnosed cancer among South African women and a major cause of cancer-related deaths worldwide. Photodynamic therapy (PDT) is an alternative cancer therapy that uses photochemotherapeutic agents, known as photosensitizers. Drug-delivery nanoparticles are commonly used in nanomedicine to enhance drug-therapeutic efficiency. Targeted or selective approaches used during cancer treatment determine the efficacy and outcome of the therapy. In order to enhance specificity and targeting and obtain better treatment options for cancer, novel modalities are currently under development. Photodynamic therapy has the potential to eradicate cancer, and combination therapy would yield even greater outcomes. This study evaluated the photodynamic effects following treatment with 0.3 mM multiple particles delivery complex (MPDC) and irradiated with a laser fluence of 10 J/cm2 using a 680 nm diode laser in a breast cancer cell line (MCF-7).

Methods

Cell damage was assessed by inverted light microscopy for cell morphology; the Apoptox-Glo triple assay was used for cell viability, caspase activity and identification of cytodamage markers; flow cytometric analysis for cell death pathways and mitochondrial membrane potential; the enzyme linked immunosorbent assay (ELISA) for cytochrome C release; and real-time reverse transcriptase polymerase chain reaction (RT-PCR) array for gene expression.

Results

Laser activated-MPDC induced a significant change in morphology of PDT-treated cells, with the appearance of apoptotic like morphological features. An increase in cytotoxicity, caspase activity, cell depolarization and cytochrome C release were identified in PDT-treated cells. Finally, the upregulation of BAX, BCL-2, CASP-2 and ULK-1 genes was observed.

Conclusion

The MPDC yielded a successful and stable hybrid agent with potent photodynamic abilities.

Keywords: Cancer, Photodynamic effects, Nanomedicine, Cell damage, Cell death

Acknowledgements

Ivan Tynga

Conflicts of Interest

The author declares no conflict of interest.

References

1. Mfouo Tynga I, Houreld N and Abrahamse H. (2018). Evaluation of cell damage induced by irradiated Zinc-Phthalocyanine-gold dendrimeric particles in a breast cancer cell line. Biomedical Journal. 41 (2018): 254-264, https://doi.org/10.1016/j.bj.2018.05.002 Impact factor 3.94.
2. Mfouo Tynga, I. and Abrahamse, H. (2018) Nano-Mediated Photodynamic Therapy for Cancer: Enhancement of Cancer Specificity and Therapeutic Effects. Nanomaterials. 2018, 8, 923; doi:10.3390/nano8110923 Impact Factor 3.5

> P158. Poster

Symposium PCHEM-3 Nanobioplasmonics (Belinda Heine)

HYBRID NANOPARTICLES FOR THERAPY AND DIAGNOSIS

Authors: Jesús M. de la Fuente[1]
Presenting Author: Jesus M. de la Fuente
1) Instituto de Ciencia de Materiales de Aragón, CSIC-Universidad de Zaragoza & CIBER-BBN, Spain.

In the last decades, inorganic nanoparticles have been steadily gaining more attention from scientists from a wide variety of fields such as material science, engineering, physics or chemistry. The very different properties compared to that of the respective bulk, and thus intriguing characteristics of materials in the nanometre scale, have driven nanoscience to be the centre of many basic and applied research topics. Moreover, a wide variety of recently developed methodologies for their surface functionalization provide these materials with very specific properties such as drug delivery and circulating cancer biomarkers detection. In this talk we describe the synthesis and functionalization of magnetic and gold nanoparticles as therapeutic and diagnosis tools against cancer.

Gold nanoprisms (NPRs) have been functionalized with PEG, glucose, cell penetrating peptides, antibodies and/or fluorescent dyes, aiming to enhance NPRs stability, cellular uptake and imaging capabilities, respectively. Cellular uptake and impact was assayed by a multiparametric investigation on the impact of surface modified NPRs on mice and human primary and transform cell lines. Under NIR illumination, these nanoprobes can cause apoptosis. Moreover, these nanoparticles have also been used for optoacoustic imaging, as well as for tumoral marker detection using a novel type of thermal ELISA and LFIA nanobiosensor using a thermosensitive support.

> IL333. Invited Lecture
Symposium PCHEM-4 Upconverting nanoparticles (Julia Pérez-Prieto)

OPTICAL PROPERTIES OF DIFFERENT TYPES OF LUMINESCENT NANOCRYSTALS AT THE ENSEMBLE AND SINGLE EMITTER LEVEL

Authors: Florian Weigert[1], Florian Frenzel[1], Christian Würth[1], Katrin Hoffmann[1], Irina Martynenko[1], Lorena Dhamo[1], Ute Resch-Genger[1]
Presenting Author: Ute Resch-Genger
1) Federal Institute for Materials Research and Testing (BAM)

Applications of luminescent nanomaterials like semiconductor nanocrystals (QDs) and lanthanide-based upconversion nanocrystals (UCNPs) in the life sciences such as bioimaging studies or their use as reporter in assays call for a correlation of the photoluminescence (PL) properties of these nanomaterials on ensemble and single particle levels. This is particularly relevant within the context of continuously decreasing detection limits. Aiming at optimum nanomaterials for spectroscopic and microscopic applications, we examine the optical properties of QDs like II/VI QDs and cadmium-free $AgInS_2$/ZnS QDs (AIS/ZnS) and UCNPs of different chemical composition, size, and particle architecture for ensembles and single particles. This includes PL spectra, PL quantum yields (Φ_F), brightness values, blinking behavior, and PL decay kinetics. For UCNPs with their nonlinear spectrally converted PL excited by sequential multiphoton absorption, these measurements were also done as a function of excitation power density (P). Special emphasis is dedicated to the performance parameters Φ_F and brightness, that determine signal size and provide a measure for nanocrystal quality.[1-5]

Systematic studies of the excitation energy dependence (EED) [6] of the PL properties of II/VI and ternary $AgInS_2$/ZnS QDs reveal the potential of this relatively simple method for providing insights into the electronic energy structure of QDs. The intrinsic nature of the inhomogeneous broadening of the PL bands of AIS/ZnS QDs was confirmed by single particle spectroscopy.[5] By combining P-dependent integration spectroscopy and single particle measurements of UCNPs, using a new custom-made setup, consisting of different lasers, an inverted microscope, different detectors, and an AFM, we could study the P-dependent optical properties of these nonlinear emitters from ~10 W/cm^2 up to ~10^5 W/cm^2. These results provide optimum dopant ion concentrations for bioanalytical, spectroscopic, and microscopic applications of UCNP.

Acknowledgement

Financial support by grants RE1203/12-3 and RE1203/20-1 (support of F. Weigert, L. Dhamo, and F. Frenzel) from German Research Council (DFG) is acknowledged.

References

[1] Grabolle, M.; Spieles, M.; Lesnyak, V.; Gaponik, N.; Eychmüller, A.; Resch-Genger, U., *Anal. Chem* **2009**, 81, 6285-6294.
[2] Würth, C.; Grabolle, M.; Pauli, J.; Spieles, M.; Resch-Genger, U., *Nat. Prot.* **2013**, 8, 1535-1550.
[3] Kaiser, M.; Würth, C.; Kraft, M.; Hyppanen, I.; Soukka, T.; Resch-Genger, U., *Nanoscale* **2017**, 9, 10051 - 10058.
[4] Abbandonato, G.; Hoffmann, K.; Resch-Genger, U., *Nanoscale* **2018**, *10*, 7147-7154.
[5] Stroyuk, O.; Weigert, F.; Raevskaya, A.; Spranger, F.; Würth, C.; Resch-Genger, U.; Gaponik, N.; Zahn, D. R. T., *J. Phys. Chem. C* **2019**, *123* (4), 2632-2641.
[6] Martynenko, I. V.; Baimuratov, A. S.; Osipova, V. A.; Kuznetsova, V. A.; Purcell-Milton, F.; Rukhlenko, I. D.; Fedorov, A. V.; Gun'ko, Y. K.; Resch-Genger, U.; Baranov, A. V., *Chem. Mater.* **2018**, *30* (2), 465-471.

> IL3313. Invited Lecture
Symposium PCHEM-4 Upconverting nanoparticles (Julia Pérez-Prieto)

UPCONVERTING NANOPARTICLES AS REPORTERS IN ULTRASENSITIVE IMMUNOASSAYS

Authors: Tero Soukka[1]
Presenting Author: Tero Soukka
1) University of Turku, Turku, Finland.

Immunoassays are widely employed analytical tool to measure tiny concentrations of biological substances of interest from complex clinical samples. These assays generally rely on the structural specificity of antibody-antigen binding interaction and the high specific activity of the reporter system to achieve the low detection limits required. Upconverting nanoparticles (UCNPs) are an attractive choice as a photoluminescent reporter due to their unique capability to convert near-infrared excitation to visible light. The detection of upconversion luminescence (UCL) enables total elimination of autofluorescence and potentially unprecedented sensitivity. The analytical sensitivity in solid-phase immunoassays is ultimately only limited by the separation efficiency and the non-specific binding interactions of the reporter conjugate resulting in elevated level and increased variation of the assay background.

Cardiac troponin (cTn) I is a clinically significant biomarker, which is used in diagnosis of acute myocardial infarction (AMI). An ultra-low limit of detection of cTnI is required for early diagnosis of AMI. Thus, cTnI was chosen as a model analyte for an immunoassay using $NaYF_4$:Yb^{3+},Er^{3+} UCNPs as reporters. Ligands present on as-synthesized UCNPs (ca. 30 nm in diameter) were removed with acid treatment and the nanoparticles were coated with poly(acrylic acid) (PAA; Mw 2000). An anti-cTnI monoclonal antibody was conjugated covalently to PAA-coated UCNPs with carbodiimide chemistry. In the immunoassay, biotinylated anti-cTnI antibody and antibody fragment were first immobilized to streptavidin-coated microtiter wells, and cTnI calibrators and cTnI added plasma samples were incubated in the wells for 30 min and the wells were washed. Thereafter, the anti-cTnI-antibody conjugated UCNPs were incubated for 15 min in a buffer comprising unconjugated PAA, and the wells were washed four times. UCL from dry wells was measured at 525-550 nm with a dedicated upconversion microplate reader equipped with 980 nm diode laser.

The developed antibody-UCNP conjugate based immunoassay allowed highly sensitive detection of cTnI from buffer and plasma. The obtained limit of detection (blank plus three times standard deviation) was down to 0.5 ng/l and the calibration curve was linear up to cTnI concentration 50000 ng/l. The addition of unconjugated PAA to the incubation buffer of antibody-conjugated UCNPs resulted in remarkably decreased non-specific binding and increased response with cTnI calibrators. The effect was associated to the unconjugated PAA masking the non-specific interactions of antibody-UCNP conjugates with the protein coated microtiter well surface. Since the measured assay background without analyte was still over ten times higher than the background obtained by excluding the antibody-UCNP conjugate from the assay, there is still potential for further improvements through additional countermeasures against the non-specific binding interactions.

> IL332. Invited Lecture
Symposium PCHEM-4 Upconverting nanoparticles (Julia Pérez-Prieto)

THE DEVELOPMENT OF SENSING PLATFORMS FOR THE DETECTION OF SUBSTRATES USING PROTEIN BOUND UPCONVERTING PHOSPHORS

Authors: Louise Natrajan[1], Letitia Burgess[1], Peter Harvey[1], Sam Hay[1], Alex Jones[1]
Presenting Author: Louise Natrajan
1) The University of Manchester

Introduction

Current biological imaging and sensing methods commonly utilise organic dyes fluorescent imaging agents. However they suffer from problems such as broad optical profiles, competitive autofluorescence of biological molecules and photobleaching, which limit their effectiveness in biological applications. Lanthanide doped upconverting phosphors (UCPs) on the other hand have emerged as a new class of compounds for use in biological applications, as they offer improved chemical stability and excitation is achieved using near infrared light; a region in which biological samples are silent and a wavelength capable of penetrating deeper into the tissue.

Methods

Since proteins and enzymes play a crucial role in key biological processes including those involved in disease, we have been exploring the use of upconverting luminescent lanthanide doped phosphors (UCPs) to detect a range of biological co-factor containing molecules by exploiting the spectral overlap of the lanthanide visible emission to induce an inner filter or luminescence energy transfer reponse.

Results and Discussion

We demonstrate the use of these UCPs to detect the concentration level and function of biologically important analytes, that include enzymes and key disease biomarkers (here, pentaerythritol tetranitrate reductase, glucose oxidase, vitamin B_{12}, and cytochrome c). By tailoring the absorption profile of the biomolecule cofactors to the UCP emission, we have been able to show that a wide-range of analytes at analytically useful concentrations can be sensed, thereby opening up potential scope for a new class of luminescent based biosensors that function based on luminescence energy transfer. Furthermore, we have covalently attached these biomolecules to the UCPs and demonstrate the ability of these systems to reversibly monitor the addition of enzyme substrates via repeat oxidation and reduction of the flavin cofactor in the enzyme pentaerythritol tetranitrate reductase.

References

1) A. Sedlmier and H. Gorris, *Chem. Soc. Rev.*, 2015, **44**, 1526 – 1560
2) P. Harvey, C. Oakland, M. D. Driscoll, S. Hay and L. S. Natrajan, *Dalton Trans.*, 2014, **43**, 5265-5268;
3) S. Wilhelm, M. de Barrio, J. Heiland, S. F. Himmelstoβ, J. Galbán, O. S. Wolfbeis and T. Hirsch, *ACS. Apl. Mater. Interfaces*, 2014, **6**, 15427-1543

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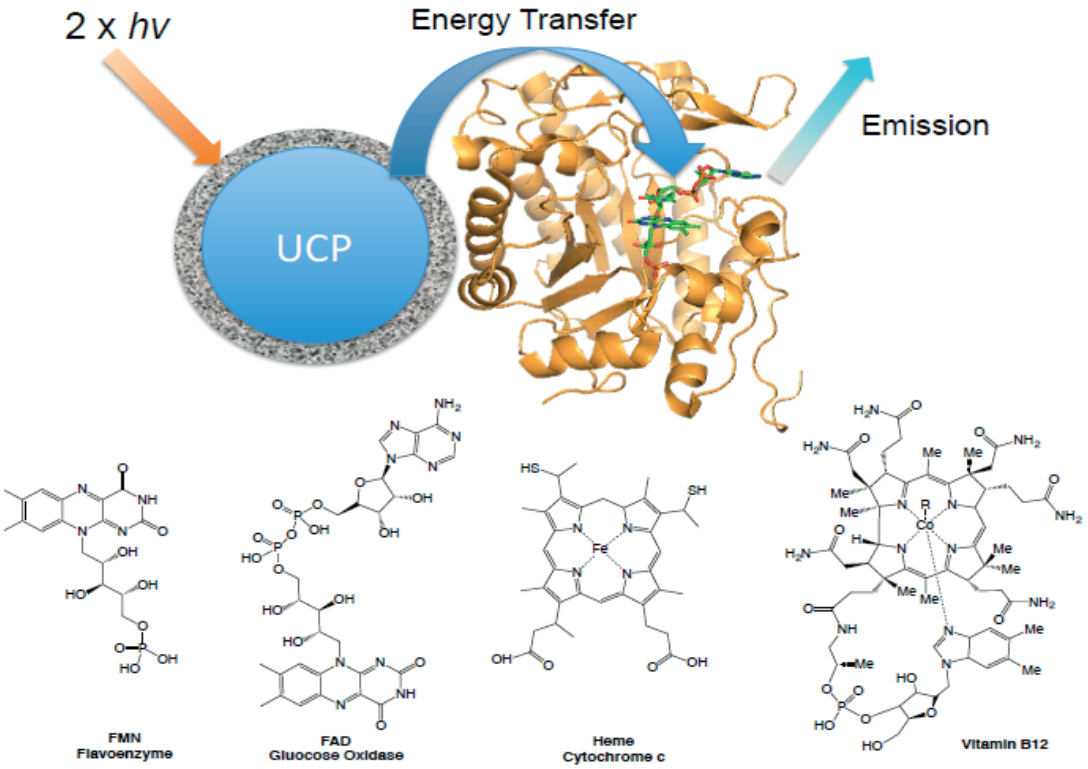
2 x hv
Energy Transfer
Emission
UCP
FMN
Flavoenzyme
FAD
Gluocose Oxidase
Heme
Cytochrome c
Vitamin B12

> IL330. Invited Lecture
Symposium PCHEM-4 Upconverting nanoparticles (Julia Pérez-Prieto)

FRET WITH UPCONVERTING NANOPARTICLES – FROM FUNDAMENTAL UNDERSTANDING TO APPLIED BIOSENSING

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The use of upconversion nanoparticles (UCNPs) as donors in Förster resonance energy transfer (FRET) provides distinct advantages for biosensing. UCNPs can be excited in the near-infrared (NIR), which results in strongly reduced autofluorescence from biological samples and higher signal-to-noise ratios, together with lower phototoxicity compared to UV or blue light excitation.[1–3] Several studies have assessed FRET efficiencies of UCNPs with organic dye or quantum dot acceptor, and it was postulated that only lanthanide ions close to the surface were participating in energy transfer.[4–7] Because these ions are close to the surface, they are prone to quenching by OH-groups in aqueous solutions, which, in turn, would decrease the FRET efficiency and lead to less sensitive UCNP-based FRET biosensors.[8]

In this study we investigated the role of erbium ions (Er^{3+}) participating in FRET by studying steady-state and time-resolved photoluminescence (PL) of both UCNP donors and cyanine dye acceptors (Cy3.5 and Cy5.5), which were present at different distances from the UCNP surface. A $NaYF_4$ nanoparticle doped with Yb^{3+} and Er^{3+} was either silanized or modified with poly(acrylic acid) (PAA) for further bioconjugation with single-stranded (ss) DNA. The cyanine dyes (conjugated to complementary ssDNA) were brought close to the UCNPs' surface by designing hybridization-assays in different environments such as H_2O and D_2O. In both H_2O and D_2O the addition of Cy-ssDNA led to a strong sensitized emission of Cy3.5 and Cy5.5. Since excitation at 980 nm did not directly excite the dyes but only the UCNPs, sensitized dye emission must originate from energy transfer from the UCNP donor. Control measurements revealed a strong distance dependence of the energy transfer with minor contributions from radiative energy transfer. PL lifetime analysis revealed that apparent FRET efficincies in D_2O were only slightly larger than in H_2O, in contrast to our expectations related to the strong surface quenching of UCNPs in H_2O. The optimal UCNP-dye FRET system was used to construct a homogeneous sandwich hybridization assay for the detection of the micro-RNA miR-20a with a limit of detection of 1.2 nM.

References

[1] Haase, M.; Schäfer, H. *Angew. Chem. Int. Ed*. **2011**, 50, 5808–5829, [2] Hilderbrand, S. A.; Shao, F.; Salthouse, C.; Mahmood, U.; Weissleder, R. *Chem. Commun*. **2009**, 4188–4190, [3] Hemmer, E.; Benayas, A.; Légaré, F.; Vetrone, F. *Nanoscale Horiz*. **2016**, 1, 168–184, [4] Dukhno, O.; Przybilla, F.; Collot, M.; Klymchenko, A. S.; Pivovarenko, V.; Buchner, M.; Muhr, V.; Hirsch, T.; Mely, Y. *Nanoscale* **2017**, **9**, 11994-12004, [5] Bednarkiewicz, A.; Nyk, M.; Samoc, M.; Strek, W. *J. Phys. Chem. C* **2010**, 114, 17535–17541, [6] Muhr, V.; Würth, C.; Kraft, M.; Buchner, M.; Baeumner, A. J.; Resch-Genger, U.; Hirsch, T. *Anal. Chem.* **2017**, 89, 4868–4874, [7] Bhuckory, S.; Hemmer, E.; Wu, Y.-T.; Yahia-Ammar, A.; Vetrone, F.; Hildebrandt, N. *Eur. J. Inorg. Chem.* **2017**, 5186–5195, [8] Arppe, R.; Hyppänen, I.; Perälä, N.; Peltomaa, R.; Kaiser, M.; Würth, C.; Christ, S.; Resch-Genger, U.; Schäferling, M.; Soukka, T. *Nanoscale* **2015**, 7, 11746–11757.

> IL334. Invited Lecture
Symposium PCHEM-4 Upconverting nanoparticles (Julia Pérez-Prieto)

BIOCOMPATIBILITY ASSESSMENT OF UP- AND DOWN-CONVERTING NANOPARTICLES: IMPLICATIONS OF INTERFERENCES WITH IN VITRO ASSAYS

Authors: Ivana Vinković Vrček[1], Barbara Pem[1], Krunoslav Ilić[1], Ivan Pavičić[1], Daniel González-Mancebo[2], Nuria O. Nuñez[2], Ana Isabel Becerro[2], Manuel Ocaña[2]
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Efficacy, quality and safety assessment of nanoparticles (NPs) is crucial during their design and development for biomedicine. One of the prerequisite steps during this evaluation is *in vitro* testing that employs cell-based assays not always validated and well-adapted for NPs. Interferences with *in vitro* assays may arise due to the nano-related optical, oxidative, fluorescent, surface and catalytic properties of NPs. Thus, proper validation of each assay system has to be performed for each NP type. The unique up- and down-shifting nanoparticles (UCNPs and DSNPs, respectively) are one of the attractive NPs due to their photoluminescent properties that inspired many bioanalytical applications. Their long luminescence lifetimes and excellent photostability enable multiplexed detection in deep tissues, but translational gap between laboratory and clinics still exists due to safety concerns of therapeutic and diagnostic ("theranostic") applications of such-NPs.

This study aimed to evaluate the applicability of the most common *in vitro* cytotoxicity assays for the safety assessment of up- and down-converting lanthanide-doped NPs. Conventional cell viability tests and fluorescence-based assays for oxidative stress response were selected to determine the biological effects of UCNPs and DSNPs to diverse human cells. Comparison was performed with known silver and iron oxide NPs for verification purposes. Both the plate reader and flow cytometric measurements were examined. The obtained results indicated that all types of NPs interfered to a much lesser extent than metallic NPs. In addition, the great potential of both UCNPs and DSNPs for biomedicine was manifested due to their biocompatibility and low toxicity. In addition, biological effects and toxicity of these NPs are tissue- and cell-dependent.

Better and safer design of UCNPs and DSNPs for theranostic use should rely on integrative and interdisciplinary approach encompassing materials synthesis and research, detection instrumentation, biofunctionalization and bioassay development to toxicity testing.

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> IL329. Invited Lecture
Symposium PCHEM-4 Upconverting nanoparticles (Julia Pérez-Prieto)

THERANOSTIC APPLICATION OF UPCONVERSION NANOPARTICLES WITH TANDEM SENSITIZATION

Authors: Thomas Hirsch[1]
Presenting Author: Thomas Hirsch
1) Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg

Small, bright and colloidally stable upconversion nanoparticles (UCNPs) are desired for many theranostic applications. $NaYF_4$ with Yb^{3+}sensitization are the most common systems used so far, but these materials suffer from excitation at 980 nm. At this excitation wavelength water shows a local absorption maximum, which causes sample heating. Here, the design and synthesis of core/multi-shell monodisperse, pure hexagonal nanoparticles with high upconversion efficiency excitable at 808 nm by tandem sensitization of Nd^{3+} to Yb^{3+} is reported. These particles are surface engineered to warrant colloidal stability as well as biocompatibility. For particles with diameters in the range of 30 nm a systematic study of the upconversion efficiency influenced by doping ratios of lanthanide ions as well as by the relation of the core- to the shell-thickness allowed a better understanding of the energy transfer. Architectures consisting of a core of ~24 nm $NaYF_4$(20%Yb,0.3%Tm) with a 4 nm thick $NaYF_4$(10%Nd,10%Yb) shell revealed maximum brightness at low power excitation. These UCNPs were further modified by a mesoporous silica shell with an entrapped drug and a photo-switchable polymer to be used for the NIR light triggered drug release for controlled neural stem cell differentiation. The advantages of this system are minimized sample heating, improved penetration depth and high upconversion efficiency for an efficient drug release.

> IL335. Invited Lecture
Symposium PCHEM-4 Upconverting nanoparticles (Julia Pérez-Prieto)

UPCONVERSION NANOMATERIALS FOR PHOTOBIOLOGICAL APPLICATIONS

Authors: Nestor Estebanez[1], Laura Francés-Soriano[1], Juan Ferrera-González[1], María González-Béjar[1], Julia Pérez-Prieto[1]
Presenting Author: Julia Pérez-Prieto
1) University of Valencia, Instituto de Ciencia Molecular (ICMol), Spain

Photon upconversion occurring in strongly absorbing molecular chromophores and nanomaterials after a sequential absorption of two photons, or by means of a triplet-triplet annihilation process, is not usually an efficient process (< 10^{-8}) and requires a pulsed laser for excitation. However, the photon upconversion process is much more efficient (< 10^{-1}-10^{-3}) in lanthanide-doped nanoparticles (Ln-NPs), which consist of a crystalline host matrix (e.g., $NaYbF_4$) doped with certain. These Ln must be suitable for upconversion processes (e.g. $Yb^{3+}$and Er^{3+}) which can occur by irradiation with a low-cost continuous–wave diode laser, leading to narrow emissions at wavelengths spanning from ultraviolet to NIR.[1] The upconversion process in Ln-NPs encompasses a series of advantages, such as the depth penetration of NIR light, minimal background interference and little damage to biological samples. Ln-doped NPs are currently being studied for biological applications in view of their very low toxicity.

Ln-NPs can be prepared as water-dispersible nanomaterials for different purposes by changing the nanoparticle composition (matrix and/or dopant composition) and the functionality of the organic capping. The stability of the anchoring of the capping to the Ln-NP surface is crucial for its luminescence efficiency in aqueous solutions and, of particular interest for biological applications, for preserving its low toxicity, since bare NPs in water progressively disintegrate into their compositional ions ($F^-$is of special concern).

Examples of Ln-doped NPs functionalized with photosensitizers, fluorophores, functional polymers and a polymeric capping resistant to strongly acidic conditions [2] will be presented.

Acknowledgements

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References

[1] Francés-Soriano, L.; González-Béjar, M.; Pérez-Prieto, J. in Upconverting Nanomaterials: Perspectives, Synthesis, and Applications, CRC Press, 2016, pp 101-138.[2] González-Béjar, M.; Francés-Soriano, L.; Pérez-Prieto, J. Front. Bioeng. Biotechnol., 2016, 4, 47. Estebanez, N. et al. Nanoscale, 2018, 10, 12297-12301.
[2] Estebanez, N.; González-Béjar, M.; Pérez-Prieto, P. ACS Omega 2019, 4, 3012−3019. Francés-Soriano, et al. J. Chem. Mater. 2018, 30, 3677−3682. Francés-Soriano, L. et al. Nanoscale 2016, 8, 204; Francés-Soriano, L.; González-Béjar, M.; Pérez-Prieto, J. Nanoscale 2015, 7, 5140; González-Béjar, M. Et al. J. Mater. Chem. B 2014, 2, 4554; Liras, M. et al. Chem. Mater. 2014, 26, 401.

> IL336. Invited Lecture
Symposium PCHEM-5 Type-I Photooxidation processes (Maurício Baptista)

PHOTOSENSITIZED RADICAL OXIDATION REACTIONS OF ISOLATED AND CELLULAR DNA

Authors: Jean Cadet[1], J Richard Wagner[1]
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Oxygen independent reactions that involve the formation of three classes of bipyrimidine photoproducts constitute the predominant low intensity UV radiation-induced degradation pathways of both isolated and cellular DNA. These result from the direct excitation of pyrimidine bases. On the other hand, the generation of oxidation products is a minor process with the exception of UVA irradiation of cells and human skin, which leads to the formation of oxidized bases and DNA single strand breaks through photosensitized reactions. The main UVA oxidatively generated base damage was identified as 8-oxo-7,8-dihydroguanine, which is generated in model studies and also in cellular DNA by three different oxidative pathways: hydroxyl radical ((•OH), one-electron oxidation and singlet oxygen ($^1O_2$). Evidence has been provided that UVA-induced DNA damage in cells is mostly triggered by $^1O_2$, the reactive oxygen species of a type II photosensitization mechanism that selectively reacts with guanine leading to the exclusive generation of 8-oxoGua. It was also found that highly reactive •OH contributes in a minor way to the formation of 8-oxoGua together with the oxidation of pyrimidine bases and the 2-deoxyribose moiety. The formation of •OH by a type I photosensitization mechanism likely involves an indirect reaction in which the excited photosensitizer undergoes charge transfer to oxygen initially giving superoxide anion radical ($O_2^{\bullet-}$). In a second step, $O_2^{\bullet-}$ transforms into hydrogen peroxide (H_2O_2) by dismutation, and H_2O_2 subsequently reacts with a transition metal ion, such as Fe^{2+} to generate •OH in a so-called Fenton like reaction. One-electron oxidation of nucleobases including reactive guanine that was extensively studied in model studies using type I photosensitizers, such 2-methyl-1,4-naphthoquinone and riboflavin, is less likely to occur in cellular DNA since it requires contact between the bases and the triplet excited sensitizer. Photolysis of cellular DNA with high intensity 266 nm laser pulses constitutes a suitable way to generate reactive radical cations through bi-photonic ionization of the bases and to investigate the mechanism by quantitative analysis of the photoproducts using HPLC-ESI-MS/MS. Thereby, 8-oxoGua was found to be the predominant product formed at the expense of other base oxidation products, which underlines the efficiency of charge transfer with preferential hole trapping by guanine bases. Other identified lesions include intra- and interstrand crosslinks together with DNA-protein crosslinks that arise as for 8-oxoGua by nucleophilic addition to the guanine radical cation.

References

MS Baptista et al, Photochem Photobiol 2017, 93:912-9
J Cadet & T Douki, Photochem Photobiol Sci 2018, 17:1816-41
J Cadet et al, Free Radic Biol Med 2017, 107:13-34
P Di Mascio et al, Chem Rev 2019, 119:2043-86
J Cadet et al, Photochem Photobiol 2019, 95:59-72

> IL340. Invited Lecture
Symposium PCHEM-5 Type-I Photooxidation processes (Maurício Baptista)

ONE-ELECTRON OXIDATION OF NUCLEOBASES AND AMINO ACIDS PHOTOSENSITIZED BY PTERIN

Authors: Carolina Lorente[1], M. Laura Dántola[1], Mariana P. Serrano[1], Sandra Estébanez[1], Lara Reid[1]
Presenting Author: Andrés H. Thomas
1) Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Universidad Nacional de La Plata (UNLP) - CONICET

Aromatic (oxidized) pterins are natural photosensitizers that accumulate in the skin of patients suffering from vitiligo, a chronic depigmentation disorder. These compounds absorb UV-A radiation, produce reactive oxygen species and photoinduce the oxidation of DNA, lipids and proteins. The photosensitizing properties of pterin (Ptr), the parent unsubstituted compound of oxidized pterins, and vitiligo-related pterin derivatives (biopterin, formylpterin and carboxypterin) have been studied using purine and pyrimidine nucleotides as substrates. The predominant mechanism involved in the pterin-photosensitized oxidation of these compounds is the type I, and is initiated by a one-electron transfer from the nucleotide to the triplet excited state of pterins.[1,2]

The fate of the organic radicals is strongly dependent on chemical nature of the nucleobase and the presence of dissolved O_2. In studies performed with 2'-deoxyguanosine 5'-monophosphate (dGMP) in air-equilibrated solutions, we have identified many products containing the oxidized guanine moiety, whereas under anaerobic conditions recombination of radicals takes place and dGMP is not consumed.[2,3] In the case of thymine (Thy), in air-equilibrated solutions, the products can be explained taking into account the typical reactions of the Thy radical cation ($Thy^{\bullet+}$); but under anaerobic conditions, coupling of radicals takes place yielding a covalent adduct Ptr-Thy.[4] This compound, which has the intact pterin moiety and retains some of its photochemical properties, can be also formed using DNA as a substrate.[5]

Type I photooxidation is also the predominant mechanism in the Ptr-photosensitized degradation of free amino acids and proteins.[6] Studies carried out using albumin and ubiquitin as a model protein suggested the oxidation of several amino acids and the binding of Ptr to the protein to yield a fluorescent product.[7]

References

1. G. Petroselli, M. L. Dántola, F. M. Cabrerizo, A. L. Capparelli, C. Lorente, E. Oliveros, A. H. Thomas, *J. Am. Chem. Soc.* **130**, 3001–3011 (2008).
2. M. P. Serrano, C. Lorente, C. D. Borsarelli, A. H. Thomas, *ChemPhysChem.* **16**, 2244–2252 (2015).
3. M. P. Serrano, S. Estébanez, M. Vignoni, C. Lorente, P. Vicendo, E. Oliveros, A. H. Thomas, *New J. Chem.*, **41**, 7273-7282 (2017).
4. M. P. Serrano, M. Vignoni, C. Lorente, P. Vicendo, E. Oliveros, A. H. Thomas, *Free Radical Biol. Med.*, **96**, 418–431 (2016).
5. S. Estébanez, C. Lorente, M. Gaspar Tosato, M. A. Miranda, M. L. Marín, V. Lhiaubet-Vallet, A. H. Thomas, *Dyes Pigm.* **160**, 624–632 (2019).
6. A. H. Thomas, M. P. Serrano, V. Rahal, P. Vicendo, C. Claparols, E. Oliveros, C. Lorente, *Free Radical Biol. Med.* **63**, 467–475 (2013).
7. L. O. Reid, E. A. Roman, A. H. Thomas, M. L. Dantola, *Biochemistry*, **55**, 4777–4786 (2016).

> IL337. Invited Lecture
Symposium PCHEM-5 Type-I Photooxidation processes (Maurício Baptista)

EXPLOITING CHOLESTEROL AS A NATURAL MECHANISTIC PROBE FOR PHOTOSENSITIZED OXIDATION REACTIONS

Authors: Albert Girotti[1], Witold Korytowski[2]
Presenting Author: Albert Girotti
1) Medical College of Wisconsin 2) Jagiellonian University

The monounsaturated lipid, cholesterol (Ch), is abundant in plasma membranes of mammalian cells and in plasma lipoproteins. Unlike natural phospholipids, Ch exists as a single molecular species and its relatively few oxidation products are readily separated and characterized. Two early products/intermediates can be used as mechanistic reporters in photodynamic reactions: (i) 3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide (5α-OOH), which is generated exclusively by singlet oxygen ($^1O_2$) attack on Ch, and (ii) 3β-hydroxycholest-5-ene-7α/7β-hydroperoxide (7α/7β-OOH), which arises via abstraction of an allylic H from C7 of Ch, e.g. by a strongly oxidizing excited state sensitizer or a strong downstream oxidant such as hydroxyl radical (HO·). Initial 7α/7β-OOH formation by photodynamic action in a biomembrane system would signify Type I (free radical) primary photochemistry, whereas 5α-OOH formation would signify Type II ($^1O_2$) photochemistry. Separation and high sensitivity detection of these positional cholesterol hydroperoxide (ChOOH) species is readily accomplished by reverse-phase HPLC with mercury cathode electrochemical detection [HPLC-EC(Hg)] developed by the authors. Early kinetic measurement of 5α-OOH or 7α/7β-OOH during membrane photooxidation is crucial because light-independent secondary reactions (chain-peroxidation via one-electron reduction of either ChOOH) would itself give rise to 7α/7β-OOH and possibly 5α-OOH via peroxyl radical disproportionation. This is a relatively novel and straightforward approach for distinguishing Type I *vs.* Type II reactions which can be applied to various photosensitizers in model or cellular membrane systems. Experimental examples of employing this ChOOH-based approach will be described and discussed. Supported by NIH grants CA72630, TW001386, and CA70823 (to A.W.G.) and NCN grant 2017/26/M/NZ3/01232 (to W.K.).

> IL339. Invited Lecture

Symposium PCHEM-5 Type-I Photooxidation processes (Maurício Baptista)

INTERFACIAL STRATEGIES TO STUDY REACTIVE OXYGEN INTERMEDIATES PRODUCED USING PHOTOSENSITIZATION

Authors: Alexander Greer[1,2]
Presenting Author: Alexander Greer
1) Brooklyn College of the City University of New York 2) The Graduate Center of the City University of New York

In this presentation we discuss the production and behavior of reactive oxygen intermediates at air-solid interfaces as well as air-water interfaces. Results of one study show that photosensitized organic peroxide homolysis at an air-solid interface proceeds through a triplet energy transfer to a repulsive O-O orbital. This conclusion is supported by a relatively short ~7 Å sensitizer-to-peroxide distance achieving the highest percent homolysis. Results of a second study show alkoxy radical reactions favor hydrogen abstraction in protic media or at the air-solid interface of silica particles. This is shown by alcohol formation in proctic media and lack of alcohol formation in non-protic media. Results of a third study show 'ene' reactions of singlet oxygen at an air-water interface to be regioselective with long-chain prenylsurfactants. The reaction proceeds through an unsymmetrical and synchronous attack by singlet oxygen on the π bond. Desolvated methyl groups are more prone to the 'ene' reaction causing long-chain surfactants to waste less airborne singlet oxygen by physical quenching. The above results demonstrate the utility of interfacial strategies for reactive oxygen intermediate analysis.

> IL338. Invited Lecture
Symposium PCHEM-5 Type-I Photooxidation processes (Maurício Baptista)

CONTACT-DEPENDENT PHOTOSENSITIZED OXIDATION WITHIN MEMBRANES AND THE EFFICIENCY OF PDT PHOTOSENSITIZERS

Authors: Mauricio S. Baptista[1], Thiago T. Tasso[2]
Presenting Author: Mauricio Baptista
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Introduction
Type I and Type II photosensitized oxidations are the main reactions in Photodynamic Therapy (PDT). Several tissues and cellular targets are responsible for the PDT response, but damage in membranes is key to modulate the mechanism as well as the overall efficiency of cell death.[1] Membrane leakage needs PS sacrifice through contact-dependent reactions, forming lipid-truncated aldehydes.[2] In this presentation we will show that photobleaching, when caused by a direct-contact reaction between the PS and a biological target (lipid double bond) correlates with the increase in PS efficiency.

Methods
Mg(II) porphyrazines (MgPzs) with different peripheral groups having electron-donating and electron-withdrawing groups, were synthesized and purified. Several spectroscopic and microscopic techniques were used to characterize efficiency and mechanism of photobleaching as well as correlate photobleaching with the efficiency of membrane rupture.

Results and Discussions
The mechanism of photobleaching of MbPzs is not related with oxidation by singlet oxygen, but instead to an electron abstraction from a neighbor molecule. By comparing the efficiency of membrane rupture by PSs with different electron-deficient fluorinated side groups, we showed that the higher the rate of photobleaching, which occurs because of a redox reaction with the lipid double bond, the faster the rate of membrane leakage. **Conclusions**: Our results indicate that the efficiency of membrane damage correlates with the efficiency of PS photobleaching, and consequently, PS regeneration should be exploited as an effective tool to developed improved PDT photosensitizers.

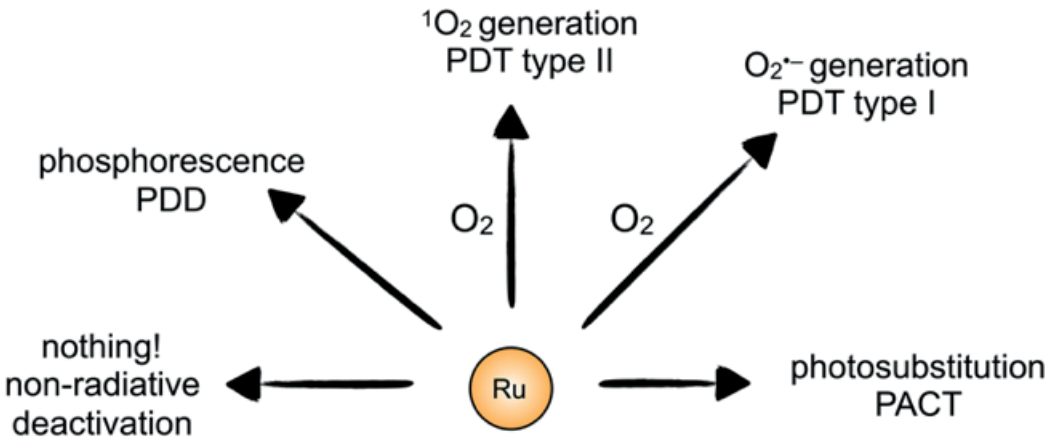
References
1. I Bacellar, et al. Photodynamic efficiency: from molecular photochemistry to cell death. *Int. J. Mol. Sci.* **2015**, 16, 20523.
2. I Bacellar, et al. Photosensitized membrane permeabilization requires contact-dependent reactions between photosensitizer and lipids, *J. Am. Chem. Soc.* **2018**, 140, 9606.

> IL342. Invited Lecture
Symposium PCHEM-5 Type-I Photooxidation processes (Maurício Baptista)

BEYOND PDT TYPE I AND II: MOLECULAR MECHANISMS OF PHOTOACTIVATED CHEMOTHERAPY

Authors: Sylvestre Bonnet[1]
Presenting Author: Sylvestre Bonnet
1) Leiden University

In PhotoDynamic Therapy (PDT), phototoxicity is generated at the place of light irradiation as a result of energy or electron transfer from the excited photosensitizer to the dioxygen present in the irradiated tumor. On the contrary, in Photo-Activated Chemotherapy (PACT) phototoxicity appears as a consequence of a light-dependent bond breakage reaction, which generates two different photoproducts, one that contains a metal, and one that does not. In this presentation, the mechanisms underlying the photoreactivity and phototoxicity of ruthenium-based PACT compounds will be discussed, as well as the main design principles that allow for making ruthenium compounds that absorb closer to or in the red region of the spectrum.



> IL341. Invited Lecture
Symposium PCHEM-5 Type-I Photooxidation processes (Maurício Baptista)

REVISITING THE MECHANISM OF BIOMOLECULES PHOTOOXIDATION AS SENSITIZED BY RIBOFLAVIN IN RELATION TO HUMAN HEALTH AND FOOD QUALITY

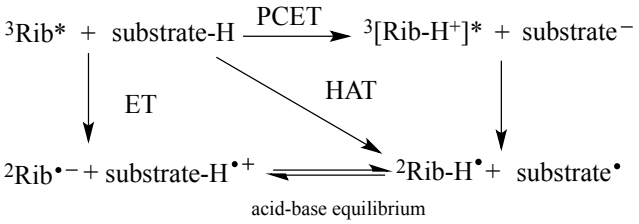
Authors: Daniel Rodrigues Cardoso[1]
Presenting Author: Daniel R. Cardoso
1) São Carlos Institute of Chemistry, University of São Paulo

Riboflavin (vitamin B2), is an efficient photosensitizer inducing oxidative damage to light-exposed tissue and food by substrate-dependent mechanisms, for which protection of human health is offered by specific nutrients. Typically, biomolecules like proteins and lipids in expose tissue or food are insensitive to direct effects of UVA and visible light due to lack of absorbing bands in the UVA-visible region of the electromagnetic spectrum. However, in the presence of riboflavin which exhibit two intense absorption bands in the UVA (375 nm) and Visible region (446 nm) of the electromagnetic spectrum, biomolecules oxidation may be driven by riboflavin Type I or II photosensitization reaction. The Type I photooxidation is a radicaloid-type mechanism in which triplet-excited riboflavin directly oxidise the biomolecules leading to formation of free radicals, whereas the Type II photooxidation proceeds by energy transfer from triplet-excited riboflavin to oxygen forming the reactive singlet-excited oxygen which may further oxidise sensitive structures and biomolecules.

The analysis of the thermodynamic and kinetic data, together with product analysis, clearly demonstrate that the mechanism behind photooxidation of biomolecules sensitised by riboflavin is strongly substrate dependent. For oxidation of a reducing substrate by triplet riboflavin, occurring as part of the Type I photosensitization, two limiting mechanisms have been recognised, electron transfer and hydrogen atom transfer. As seen from the collected specific rate constants for a number of biomolecules, substrates which reduces triplet riboflavin usually displays bimolecular rate constants falling in two separated groups ranging from 10^8 to 10^9 L mol^{-1} s^{-1} and ranging from 10^5 to 10^7 L mol^{-1} s^{-1} inviting further mechanistic speculations.

Thus, the importance of the Type II reaction mechanism may, however, be overestimated for biological systems, since protein amino acid side chains and amino acids react with triplet-excited riboflavin with similar rates through proton-coupled electron transfer or through electron transfer leading to protein radicals. Some of these protein radicals are rather long-lived and may abstract hydrogen atoms from lipids initiating free radical lipid oxidation. Type I photooxidation operating through a free radical mechanism may accordingly be more important for riboflavin in biological systems, except for air-saturated high-lipid systems where singlet oxygen oxidation of unsaturated lipids and cholesterol is favoured. For Type I riboflavin photooxidation, electron transfer and step-wise proton-coupled electron transfer reactions (k ranging from 10^8 to 10^9 L mol^{-1} s^{-1}) are faster than hydrogen atom transfer reactions (k ranging from 10^5 to 10^7 L mol^{-1} s^{-1}) and will accordingly dominate for most conditions.

Acknowledges: FAPESP (17/01189-0) and CNPq (306491/2015-0)



Scheme 1. Thermodynamic cycle for the electron transfer (ET), hydrogen atom transfer (HAT), and stepwise proton-coupled electron transfer (PCET) processes for reduction of triplet-excited riboflavin by biomolecules.

> OC127. Oral Communication
Symposium PCHEM-5 Type-I Photooxidation processes (Maurício Baptista)

EFFECT OF PH ON PHOTOINDUCED RADICAL REACTIONS BETWEEN AMINO ACIDS AND CHROMOPHORES OF HUMAN EYE LENS

Authors: Yuliya Zhuravleva[1, 2], Peter Sherin[1, 2]
Presenting Author: Yuliya Zhuravleva
1) International Tomography Center, SB RAS 2) Novosibirsk State University

Human eye lens contains low-molecular mass compounds, kynurenine (KN) and its derivatives, which absorb in UV-A region (315-400 nm) and protect eye tissues from harmful Sun irradiation. KNs are effective molecular UV filters but due to their intrinsic instability they form different products within the tissue. Some of these products are effective photosensitizers able to generate reactive triplet states under UV-A light. Reactions of these triplets with aromatic amino acid residues (mainly tryptophan and tyrosine) results in the formation of free radicals within eye lens, which subsequent reactions inevitably change the eye lens proteins, leading to their coloration and aggregation. Accumulation of different modifications within an individual lifetime may lead to the formation of cataracts, which molecular mechanisms are still weakly studied.

Oxidative stress is the necessary condition for the cataract development. Though the origin of oxidative stress formation in a healthy lens is unclear, it could be followed by acidosis, a shift of cell pH to lower values. In this work we studied the influence of pH on the photoinduced radical reactions between amino acids and kynurenic acid (KNA), one of the most effective photosensitizer of the human eye lens.

Steady-state and nanosecond laser flash photolysis, high performance liquid chromatography (HPLC) and mass spectrometry (MS) were main methods of this work.

Time-resolved optical experiments have shown that KNA radical exhibits pKa value 5.5 that is close to physiological values within a healthy lens. Therefore, it could be expected that even a slight acidification of cell environment could affect the mechanisms of photoinduced reactions. To study the influence of pH, the aqueous solutions of KNA in the presence of Trp or Tyr were irradiated by UV-A light at different pH. HPLC-MS analysis of photolysed samples have shown a decrease of decomposition yield of reagents with the lowering of pH in the case of Trp and no pH influence in the case of Tyr. In all experiments no changes in the composition of formed products were observed; only a decrease of their abundances at low pH. This indicates an increase of the restoration of KNA and Trp at low pH values that should be assigned to an increase of the rate of back electron transfer reaction between KNA and Trp radicals due to protonation of both radicals. It should be emphasized that the lowering of pH also decreases the yield of Trp dimeric forms – protein cross-links recently found in living systems.

Analogous decrease of photodegradation yield and of accumulation of covalently linked products was observed with lysozyme. This clearly shows that acidification of cell environment could decrease the amount of KNA-photoinduced modifications of eye lens proteins and minimize the total damage to the eye lens tissue.

This work was financially supported by Russian Science Foundation (project 18-73-10014).

> OC128. Oral Communication
Symposium PCHEM-5 Type-I Photooxidation processes (Maurício Baptista)

ANTIOXIDANT ACTION OF RESVERATROL IN THE PREVENTION OF GUANINE ONE-ELECTRON OXIDATION

Authors: Jael R. Neyra Recky[1], Maira Gaspar Tosato[1], Mariana P. Serrano[1], Andrés H. Thomas[1], M. Laura Dántola[1], Carolina Lorente[1]
Presenting Author: Carolinal Lorente
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Introduction
During the last years, the interest in Resveratrol (3,4´,5,-trihydroxystilbene, RSV) has increased due to the evidences found of its antioxidant action protecting biomolecules and cells from oxidative damage and it has been further exacerbated by the natural presence of RSV in some fruits and derivatives, especially in red wine.[1] 2'-Deoxyguanosine 5'-monophosphate (dGMP) is an essential constituent of DNA, and after one-electron oxidation, is modified causing DNA mutations. We evaluated the participation of RSV antioxidant action after one-electron oxidation of dGMP.

Methods
Kinetic analysis (HPLC-UV, UPLC-MS) during steady-state irradiation (Rayonet 3500 RPR lamp) and laser flash photolysis experiments (LP980, Edinburgh).

Results and Discussion
The obtained results clearly demonstrate that RSV is an efficient protector of dGMP during oxidation photosensitized by pterin. (Ptr). Under UV-A radiation, dGMP reacts with triplet excited state of the photosensitizer (^{3}Ptr*) to yield dGMP radical cation (dGMP$^{\bullet+}$/dGMP(-H)$^\bullet$), which in the absence of RSV and in the presence of O_2 undergoes oxidation.[2] In this work we confirmed that RSV reacts with both ^{3}Ptr* and dGMP(-H)$^\bullet$, with diffusion-controlled limit behavior ($k_{^3Ptr^*}$ = 4.94x10^9 M^{-1}s^{-1} and $k_{dGMP(-H)^\bullet}$ = 1.2x10^9 M^{-1}s^{-1}, respectively). However, due to the different lifetimes values of the involved species, *i.e.* dGMP(-H)$^\bullet$ (>100 ms) and ^{3}Ptr* (~6 ms), at low concentration of RSV, the antioxidant reacts significantly only with dGMP(-H)$^\bullet$, recovering the nucleotide and preventing its further oxidation. As RSV is a sacrificial molecule, after reaction with dGMP(-H)$^\bullet$, RSV radicals formed are latter oxidized, losing the antioxidant capacity.

RSV is recognized as a scavenger of Reactive Oxygen Species (ROS). During Ptr-photosensitized reactions, $O_2^{\bullet-}$ is formed and, in consequence H_2O_2 is detected in the solutions as a product. The addition of RSV does not affect the final H_2O_2 concentration, suggesting that RSV does not reacts with $O_2^{\bullet-}$ in the experimental conditions used. Moreover, the corresponding bimolecular rate constant (k_{H2O2}) for the reaction between RSV and H_2O_2 was evaluated, and a value of 1.16 (± 0.07) M^{-1}s^{-1} was obtained. This latter result discards any reaction between RSV and H_2O_2 in the current experimental conditions.

Conclusions
Considering the results presented here, we demonstrate that RSV is an efficient inhibitor of dGMP oxidation during one-electron oxidation. The antioxidant mechanism involves the reaction between RSV and the nucleotide neutral radical, dGMP(-H)$^\bullet$, to recover the native nucleotide and prevent its permanent damage, which is the main causes of carcinogenic lesions initiation. In the absence of RSV, and after one-electron oxidation, dGMP is irremediably oxidized, and if the damage occurs in dGMP located in DNA molecules, the consequences can be as serious as mutations and subsequent carcinogenic lesions.

Continued

Acknowledgements
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Conflicts of Interest
There are no conflicts to declare

References
1. S. Pervaiz, FASEB J., 2003, 17, 1975
2. G. Petroselli, M. L. Dántola, F. M. Cabrerizo, A. L. Capparelli, C. Lorente, E. Oliveros. and A. H. Thomas., J. Am. Chem. Soc., 2008, 130, 3001

> P159. Poster

Symposium PCHEM-5 Type-I Photooxidation processes (Maurício Baptista)

PHOTOPHYSICAL AND SENSITIZING PROPERTIES OF CURCUMIN IN ORGANIC SOLVENTS AND PROTEIN COMPLEXES

Authors: Andrey Sobchuk[1], Valeri Knyukshto[1], Antonina Tretyakova[1], Aliaksandr Mikulich[1], Ihar Leusenka[1], Tatsiana Ananich[1], Ludmila Plavskaya[1], Vitaly Plavskii[1]
Presenting Author: Andrey Sobchuk
1) Institute of Physics of the NAS of Belarus

In this work, we studied the spectral polarization and time-resolved fluorescence characteristics as well as the sensitizing properties of the plant-derived compound curcumin in a number of organic solvents at the room temperature and at the liquid nitrogen temperature. It is shown that absorption and fluorescence spectra, quantum yield, fluorescence lifetime and polarization of curcumin are very sensitive to the nature of the solvent.

The fluorescence quantum yield in most solvents at *t*=20 °C is low (γ=0.02-0.15), that indicates the predominant role of the processes of nonradiative deactivation of electronic excitation (intra- and intermolecular proton transfer). Fluorescence decay curves of curcumin in organic solvents exhibit non-single-exponential behavior (two-or three-exponential fit decay) with short mean fluorescence lifetime (< τ>=0.1-0.6 ns). The rise component in fluorescence decay is observed in the protic solvents at the red edge of fluorescence spectra that may be associated with proton transfer processes in the excited state. The quantum yield of singlet oxygen strongly depends on the nature of the solvent (in the tetrachloromethane γ=0.19). The interaction between the chromophores of the bichromophore curcumin molecule is poorly pronounced, as evidenced by the absence of intramolecular energy transfer (the fluorescence polarization p≈0.47 at T=77 K is close to the maximal value p=0.50 when excited over the entire long-wavelength absorption band). At the temperature of liquid nitrogen, the phosphorescence of curcumin in ethanol and in 2-methyltetrahydrofuran is registered with a maximum of about 636 nm, its excitation spectrum corresponds to the fluorescence excitation spectrum. The phosphorescence lifetime is 4.4-5.3 ms and depends on the solvent.

Spectral characteristics of curcumin and its stability greatly changed upon formation of complexes with protein molecules. For the first time, experimental evidence of the presence of inductive-resonance energy transfer for electronic excitation of tryptophanyl→curcumin has been obtained. However, it was established that quenching of tryptophanyl fluorescence upon incorporation of curcumin is due not only inductive resonance energy transfer, but the charge transfer processes.

The ability of curcumin to penetrate cells and cause their death when exposed to light is shown. A determining role in the mechanism of cell death play radical processes (type I reaction).

> P160. Poster

Symposium PCHEM-5 Type-I Photooxidation processes (Maurício Baptista)

TYROSINE DIMER, A COMMON OXIDATIVE LESION OF PROTEINS, IS ABLE TO ACT AS AN INTRINSIC PHOTOSENSITIZER

Authors: Lara O. Reid[1], Mariana Vignoni[1], M. Laura Dántola[1]
Presenting Author: Andrés H. Thomas
1) Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CCT La Plata-CONICET. La Plata, Argentina.

Introduction

The tyrosine dimer (Tyr_2), a covalent bond between two tyrosines (Tyr), is one of the most important modifications of the oxidative damage of proteins. This compound is increasingly used as a marker of aging, stress and pathogenesis. At physiological pH, Tyr_2 is able to absorb radiation at wavelengths significantly present in the solar radiation and artificial sources of light. As a result, when Tyr_2 is formed *in vivo,* a new chromophore appears in the proteins. Despite the biomedical importance of Tyr_2, the information of its photochemical properties is limited due to the drawbacks of its synthesis.

Results and Discussion

We have optimized a simple, one-step method to synthesize Tyr_2, using pterin (Ptr) as a photocatalyst. Our procedure is carried out in aqueous solutions under UV-A radiation for few minutes. The purification of Tyr_2 is performed by reverse-phase chromatography. The highly pure solution obtained was used to deeper study its photochemical properties.

We have studied the photodegradation of the acid and alkaline form of Tyr_2 in aqueous solution under UV-B and UV-A radiation. In the absence of oxygen Tyr_2 is photostable. On the other hand, excitation in the presence of oxygen leads to the photodegradation of Tyr_2, yielding different products, which conserve the dimeric structure. During its photodegradation different reactive oxygen species, like hydrogen peroxide, superoxide anion and singlet oxygen ($^1O_2$), are produced. The quantum yield of $^1O_2$ production is 0.15 ± 0.05, which is similar to that obtained for free Tyr. In addition, Tyr_2 is able to sensitize the photodegradation of Tyr.

Conclusions

This study indicates that when Tyr_2 is generated in a protein structure, an intrinsic potential photosensitizer is formed, extending the active fraction of light towards the UV-A range. Therefore a product of a photosensitized process can act as a photosensitizer itself leading to further photosensitized damage, thus amplifying the harmful effects of UV radiation on biological systems.

Acknowledgements

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Conflicts of Interest

There are no conflicts to declare.

References

D. Balasubramanian, R. Kanwar, *Mol. Cel. Biochem.*, **2002**, *234/235*, 27-38.
L. O. Reid *et al.*, *Dyes Pigm.* **2017**, *147*, 67-74.
L. O. Reid *et al.*, *Photochem. Photobiol. Sci.*, in press (DOI: 10.1039/C9PP00182D)

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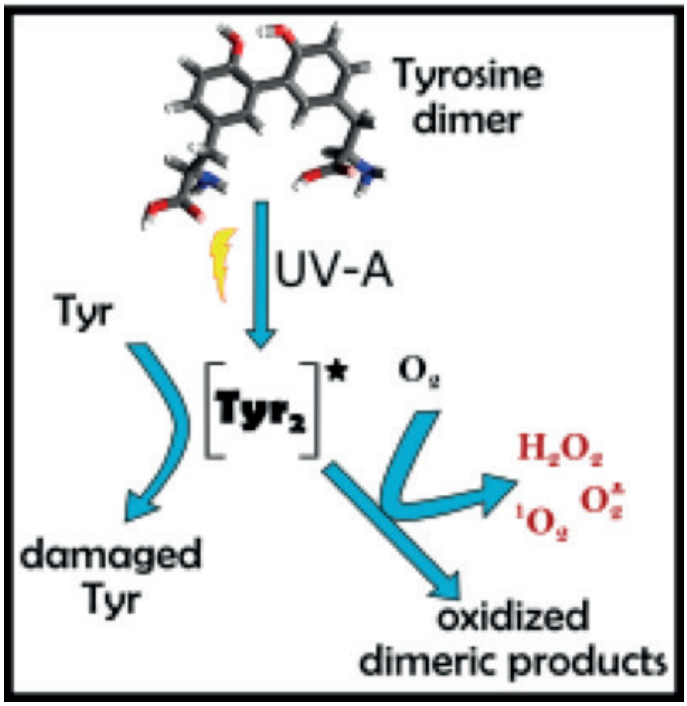
Tyrosine
dimer
UV-A
Tyr
$[Tyr_2]^*$
O_2
H_2O_2
$^1O_2$
$O_2^{\bullet-}$
damaged
Tyr
oxidized
dimeric products

> IL343. Invited Lecture
Symposium PCHEM-6 Type-II photooxidation processes (Alec Greer)

GENERATION OF SINGLET OXYGEN AND REACTIVE OXYGEN SPECIES UNDER MILD CONDITIONS

Authors: Torsten Linker[1]
Presenting Author: Torsten Linker
1) Chemistry Department, University of Potsdam

Singlet oxygen ($^1O_2$) is an important oxidant with applications in synthesis, biosciences, and material sciences.[1]It can be generated chemically or by photosensitization of ground state oxygen. The reversible reaction of acenes **1**with $^1O_2$ to endoperoxides **2**represents an attractive alternative.[2] We investigated the influence of substituents R on the kinetics of the thermolysis, which allows the release of $^1O_2$at low temperatures.[3] This approach was applied for the first intramolecular transfer of this reactive species on the basis of an oxygen anthracene sandwich complex.[4] More recently, we became interested in the release of $^1O_2$ from endoperoxides **2**by simple chemical transformations.[5]Finally, the cleavage of such compounds under acidic, basic, or even enzymatic conditions afforded reactive oxygen species, which might be used in biological applications.[6]

References

1. Recent book: *Singlet Oxygen: Applications in Biosciences and Nanosciences* (Eds. Nonell, S.; Flors, C.) Royal Society of Chemistry, Cambridge **2016**.
2. Aubry, J.-M.; Pierlot, C.; Rigaudy, J.; Schmidt, R. *Acc. Chem. Res.***2003**, *36*, 668.
3. a) Fudickar, W.; Linker, T. *Chem. Eur. J.* **2011**,*17*, 13661.
   b) Fudickar, W.; Linker, T. *J. Am. Chem. Soc.* **2012**, *134*, 15071.
4. a) Klaper, M.; Linker, T. *Angew. Chem. Int. Ed. Engl.* **2013**,*52*, 11896.
   b) Klaper, M.; Linker, T. *J. Am. Chem. Soc.***2015**, *137*, 13744.
   c) Klaper, M.; Fudickar, W.; Linker, T. *J. Am. Chem. Soc.***2016**, *138*, 7024.
5. Fudickar, W; Linker, T. *Angew. Chem. Int. Ed. Engl.***2018**, *57*, 12971.
6. Recent Review: Fudickar, W.; Linker, T. *ChemPhotoChem.***2018**,*2*, 548.

> IL344. Invited Lecture
Symposium PCHEM-6 Type-II photooxidation processes (Alec Greer)

NANOSCALE IMAGING OF AMYLOID PHOTODYNAMIC DAMAGE

Authors: Cristina Flors[1]
Presenting Author: Cristina Flors
1) IMDEA Nanoscience

The misfolding and aggregation of proteins into amyloid fibers is at the origin of many neurodegenerative disorders. In recent years, photochemical tools for blocking amyloid aggregation have been developed. Of particular interest is a thioflavin T derivative that selectively photo-oxidizes pathogenic aggregates in the presence of functional non-aggregated proteins [1]. The mechanism for selective photo-oxidation involves the enhancement of the excited-state lifetime and singlet oxygen production upon binding to specific features of amyloid aggregates due to rotational restriction. We investigate the photodynamic damage induced by this compound on model amyloid fibers using a combination of spectroscopic tools and correlative fluorescence and atomic force microscopy. Our results provide a nanoscale view of light-induced amyloid breakage, and are relevant to improve phototherapeutic strategies for amyloid-related disorders.

Reference

[1] Taniguchi, A.; Shimizu, Y.; Oisaki, K.; Sohma, Y.; Kanai, M. *Nat. Chem.* **2016**, 8, 974-982

> IL345. Invited Lecture
Symposium PCHEM-6 Type-II photooxidation processes (Alec Greer)

SINGLET-OXYGEN PHOTOSENSITIZATION IN WEAKLY-COUPLED FLOPPY COMPLEXES

Authors: Shuming Bai[1], Mario Barbatti[1]
Presenting Author: Mario Barbatti
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Triplet fusion is a class of reactions following the general internal conversion formula

$^1[^3A + ^3B] \rightarrow ^1[^1A + ^1B]$ (1)

where A + B is a molecular complex. This reaction is on the basis of important phenomena, such as singlet-oxygen photosensitization, in which a complex formed by a photosensitizer and an oxygen molecule (PS + O_2) reacts to produce $^1O_2$. The treatment of triplet fusion in a weakly-coupled floppy complex like PS-O_2 poses a great challenge for computational chemistry due to several reasons, among them, the long time scales of the process, the fact that we cannot define a unique transition state structure, and the open-shell character of the ground state.

In the last years, we have worked out a research program to address these challenges, combining conventional quantum-chemical methods with the implementation of a software for calculating spin-orbit couplings,[1] the development of a model for calculating kinetic rates for reaction (1),[2] and the proposition of proxies for efficient calculation of nonadiabatic and diabatic couplings.[3] All this research program has been specially tailored to deal with reaction (1) in weakly-coupled floppy complexes.

Taking thionucleobases and thionucleosides as prototypical PS, we have characterized their spectra,[4] intersystem crossing dynamics,[5] and the decay of their triplet state.[6-7] Finally, we have tackled the physical chemistry of reaction (1), for which we have determined singlet oxygen rates as a function of the incidence direction of the O_2 on PS.[8]

References

(1) Gao, X.; Bai, S.; Fazzi, D.; Niehaus, T.; Barbatti, M.; Thiel, W. Evaluation of Spin-Orbit Couplings with Linear-Response Time-Dependent Density Functional Methods. *J. Chem. Theory Comput.* **2017,** *13*, 515-524.
(2) Bai, S.; Barbatti, M. Divide-to-Conquer: A Kinetic Model for Singlet Oxygen Photosensitization. *J. Chem. Theory Comput.* **2017,** *13*, 5528-5538.
(3) Bai, S.; Barbatti, M. Mechanism of Spin-Exchange Internal Conversion: Practical Proxies for Diabatic and Nonadiabatic Couplings. *J. Chem. Theory Comput.* **2019**, DOI:10.1021/acs.jctc.8b00923.
(4) Bai, S.; Barbatti, M. Why Replacing Different Oxygens of Thymine with Sulfur Causes Distinct Absorption and Intersystem Crossing. *J. Phys. Chem. A* **2016,** *120*, 6342-6350.
(5) Mohamadzade, A.; Bai, S.; Barbatti, M.; Ullrich, S. Intersystem crossing dynamics in singly substituted thiouracil studied by time-resolved photoelectron spectroscopy: Micro-environmental effects due to sulfur position. *Chem. Phys.* **2018,** *515*, 572-579.
(6) Bai, S.; Barbatti, M. On the Decay of the Triplet State of Thionucleobases. *Phys. Chem. Chem. Phys.* **2017,** *19*, 12674-12682.
(7) Bai, S.; Barbatti, M. Mechanism of Enhanced Triplet Decay of Thionucleobase by Glycosylation and Rate-Modulating Strategies. *Phys. Chem. Chem. Phys.* **2018,** *20*, 16428-16436.
(8) Bai, S.; Barbatti, M. Spatial Factors for Triplet Fusion Reaction of Singlet Oxygen Photosensitization. *J. Phys. Chem. Lett.* **2017,** *8*, 5456-5460.

> IL346. Invited Lecture
Symposium PCHEM-6 Type-II photooxidation processes (Alec Greer)

SINGLET MOLECULAR OXYGEN GENERATED IN DARK PROCESS: MECHANISTIC STUDIES USING 18O-LABELED PEROXIDES AND 1O2, MASS SPECTROMETRY, AND LIGHT EMISSION MEASUREMENTS

Authors: Paolo Di Mascio[1]
Presenting Author: Paolo Di Mascio
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Singlet oxygen ($^1O_2$) is a biologically relevant reactive oxygen species capable of efficiently reacting with cellular constituents. Most of these reactions give rise to peroxides and dioxetanes, whose formation has been rationalized in terms of [4+2] cycloaddition and 1,2-cycloaddition with dienes + olefins, respectively (1).

Ultraweak chemiluminescence arising from biomolecules oxidation has been attributed to the radiative deactivation of $^1O_2$ and electronically excited triplet carbonyl products involving dioxetane intermediates. As examples, the generation of $^1O_2$ from lipid hydroperoxides, which involves a cyclic mechanism from a linear tetraoxide intermediate. The generation of $^1O_2$ via energy transfer from the excited triplet acetone arising from the thermodecomposition of dioxetane a chemical source, and horseradish peroxidase-catalyzed oxidation of 2-methylpropanal, as an enzymatic source (2).

A recent example is a pathophysiological role for $^1O_2$ in mammals through formation of an amino acid-derived hydroperoxide that regulates vascular tone and blood pressure under inflammatory conditions (3, 4). Chemically generated $^1O_2$ oxidizes the amino acid tryptophan (W) to precursors of a key metabolite called N-formylkynurenine (NFK), while enzymatic oxidation of W to NFK is catalyzed by a family of dioxygenases, including indoleamine 2,3-dioxygenase 1 (IDO1). Inflammation is associated with increased H_2O_2 and IDO1 also possesses peroxidase activity. W oxidation by IDO1 in the presence of H_2O_2 revealed that *cis*-WOOH (a tricyclic W-derived *cis*-hydroperoxide) is formed as the major product of a previously unrecognized oxidatively activated dioxygenase activity of IDO1. *cis*-WOOH is a precursor of NFK and the thermal decomposition of *cis*-WOOH also led to emission of light, characteristic of activated carbonyls (3, 4).

The approach used to demonstrate the generation of $^1O_2$ in these reactions is the use of ^{18}O-labeled peroxides / triplet dioxygen ($^{18}[^3O_2]$), the detection of labeled compounds by HPLC coupled to mass spectrometry and the direct spectroscopic detection and characterization of $^1O_2$ light emission.

The reactivity of $^1O_2$ with biomolecules, as amino acids or lipids, may generate specific stereoselective oxidation products. The elucidation of these structures and their specific targets can give important information and new horizons on the (patho)-physiological function for $^1O_2$ in mammals via formation of signaling molecules.

Acknowledgements

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References

(1) Di Mascio, P. et al. *Chem. Rev.* 119, 2043 (2019)
(2) Mano, C. M. et al. *Sci. Rep.* 4, 5938 (2014)
(3) Ronsein, G. E. et al. *Chem. Res. Toxicol.* 21, 1271 (2008)
(4) Stanley, C. P. et al. *Nature* (2019). doi.org/10.1038/s41586-019-0947-3

> IL347. Invited Lecture
Symposium PCHEM-6 Type-II photooxidation processes (Alec Greer)

ORGANIC ENDOPEROXIDES – STORING AND RELEASING SINGLET OXYGEN

Authors: Mathias Senge[1]
Presenting Author: Mathias Senge
1) Trinity College Dublin, The University of Dublin

The development of new photosensitizers for photodynamic therapy (PDT) continues unabated, both with regard to new chemical structures, formulations and as nanomaterials. Yet, less attention is given to the photochemical pathways by which singlet oxygen and other reactive oxygen species (ROS) are generated. Normally, photosensitizers exert their action in the light through generation of singlet oxygen and other ROS and their production ceases in the dark. Thus, standard PDT as a photo-therapy couples illumination and singlet oxygen generation. Yet, it is possible to do "PDT" in the dark. This requires the initial (photochemical) generation of singlet oxygen, storage in chemically stable form, and then later release via a chemical reaction. Such chemodynamic therapy (CDP) approaches can be coupled with the photochemical generation and spatial and temporal control of singlet oxygen using the reversible formation of aromatic endoperoxides.[1]

Singlet oxygen generation upon decomposition of endoperoxides at ambient temperatures represents an alternative to classical PDT, which is limited by hypoxia and reduced light penetration into cancer tissue. The concept will be illustrated using pyridone-porphyrins and heavy atom-free BODIPY-anthracene dyads, which produce triplet excited states from charge-separated excited states formed via photo-induced electron transfer.[2,3] Their interaction with molecular oxygen results in efficient $^1O_2$ generation and is accompanied by formation of highly fluorescent species due to cycloaddition of $^1O_2$ to the anthracene subunit. ROS generation from these systems in living cells induces cytotoxicity and at the same time provides fluorescent visualization of the dyads, promising new therapeutic and imaging applications.[4,5,6]

Next to offering a new approach in photomedicine these electron donor-acceptor dyads exhibit unique photochemical characteristics, notably with regard to controlling the triplet state generation by media polarity and structural factors.[7]

References

1) M. A. Filatov, M. O. Senge, *Mol. Syst. Des. Eng.* **2016**, *1*, 258.
2) M. A. Filatov, S. Karuthedath, P. M. Polestshuk, H. Savoie, K. J. Flanagan, C. Sy, E. Sitte, M. Telitchko, F. Laqaui, R. W. Boyle, M. O. Senge, *J. Am. Chem. Soc.* **2017**, *139*, 6282.
3) S. Callaghan, M. A. Filatov, E. Sitte, H. Savoie, R. W. Boyle, K. J. Flanagan, M. O. Senge, *Photochem. Photobiol. Sci.* **2017**, *16*, 1371.
4) S. Callaghan, M. O. Senge, *Photochem. Photobiol. Sci.* **2018**, *17*, 1490.
5) S. Callaghan, M. A. Filatov, H. Savoie, R. W. Boyle, M. O. Senge, *Photochem. Photobiol. Sci.* **2019**, *18*, 495.
6) M. A. Filatov, S. Karuthedath, P. M. Polestshuk, S. Callaghan, K. J. Flanagan, T. Wiesner, F. Laquaui, M. O. Senge, *ChemPhotoChem* **2018**, *2*, 606.
7) Busko, M. Jakoby, I. A. Howard, B. Richards, M. O. Senge, S. M. Borisov, A. Turshatov, *Chem. Commun.* **2018**, *54*, 1607.

> IL348. Invited Lecture
Symposium PCHEM-6 Type-II photooxidation processes (Alec Greer)

CUCURBIT[N]URIL-BASED SUPRAMOLECULAR PHOTOSENSITIZERS

Authors: José Robinson-Duggon[1,2], Nory Mariño-Ocampo[1], Felipe Andrade-Villalobos[1], Diego González[1], Jonathan Urra[1], Alexander Greer[3], Denis Fuentealba[1]
Presenting Author: Denis Fuentealba
1) Pontificia Universidad Católica de Chile 2) Universidad de Panamá 3) Brooklyn College City University of New York

Photodynamic ablation of cancer cells and/or bacterial inactivation are usually based on the generation of singlet oxygen ($^1\Delta_g$, or commonly $^1O_2$), which is a reactive oxygen species that oxidizes proteins, lipids and DNA.[1] Singlet oxygen and other ROS can be generated by an excited state photosensitizer upon irradiation in a type I or type II photoprocesses.[2] Several strategies have been proposed to improve the generation of ROS, one of them being the encapsulation of photosensitizers in supramolecular systems. Currently, the family of macrocycles called **cucurbit[*n*] urils**(CB[*n*]s, *n*= 5, 6, 7, 8, 10) has gained attention due their capacity to modify the photochemical properties of photosensitizers in a controlled fashion.[3] Moreover, different complexes can be used to switch ON or OFF the generation of singlet oxygen.[4] Our group has studied several photosensitizer-CB[*n*] complexes in the past,[5] and our current focus is on the derivatization of photosensitizers. In this context, toluidine blue O (TBO^+) was used as a "photosensitizing core" to prepare a series of derivatives with fatty acids or biomolecules. The rationale behind the derivatization was to keep the core of TBO^+, which is a good binder for CB[*n*]s, and to attach units that could favour incorporation in cancer cells. We studied the photochemistry of the derivatives in comparison with parent TBO^+using absorption/fluorescence spectroscopy and laser flash photolysis. The formation of the complexes with CB[*n*]s was characterized by fluorescence titrations and isothermal titration calorimetry (ITC). The newly synthesized molecules and their complexes show properties that are amenable for PDT applications.

References

1. Di Mascio, P., et al., *Singlet Molecular Oxygen Reactions with Nucleic Acids, Lipids, and Proteins.*Chem. Rev., 2019.**119**(3): p. 2043-2086.
2. Baptista, M.S., et al., *Type I and Type II Photosensitized Oxidation Reactions: Guidelines and Mechanistic Pathways.*Photochem. Photobiol., 2017. **93**(4): p. 912-919.
3. Robinson-Duggon, J., et al., *Potential Applications of Cucurbit[n]urils Inclusion Complexes in Photodynamic Therapy.*Isr. J. Chem., 2018. **58**(3-4): p. 199-214.
4. Robinson-Duggon, J., et al., *Supramolecular Reversible On–Off Switch for Singlet Oxygen Using Cucurbit [n] uril Inclusion Complexes.*J. Phys. Chem. C, 2017. **121**(39): p. 21782-21789.
5. Cáceres, J., et al., *Photochemical behavior of biosupramolecular assemblies of photosensitizers, cucurbit[n]urils and albumins.*Phys. Chem. Chem. Phys., 2017. **19**(3): p. 2574-2582.

> IL349. Invited Lecture
Symposium PCHEM-6 Type-II photooxidation processes (Alec Greer)

HIGHLY SENSITIVE DETECTION OF PHOTOSENSITIZED SINGLET OXYGEN WITHIN PHOTONIC CRYSTAL FIBRE

Authors: Gareth Williams[1], Sergio Adan Bermudez[1], Alexander MacRobert[2], Anita Jones[1]
Presenting Author: Anita Jones
1) University of Edinburgh 2) University College London

Highly sensitive, quantitative detection of singlet oxygen ($^1O_2$) is required for the evaluation of newly developed photosensitizers and the elucidation of the mechanisms of many processes in which singlet oxygen is known, or believed, to be involved. Arguably, the most definitive test for the presence of singlet oxygen is the measurement of its phosphorescence decay at 1270 nm. However, the extremely low intensity of this emission, coupled with the low quantum efficiency of currently available photodetectors at this wavelength, make this a challenging task.

We will describe a new approach to the direct detection of $^1O_2$ luminescence, which exploits the unique optofluidic properties of hollow-core photonic crystal fibre (HC-PCF).[1] In HC-PCF, light is trapped in the hollow core by the surrounding 2D periodic 'photonic crystal' cladding, consisting of microscopic hollow capillaries running along the entire length of the glass fibre. This allows the infiltration of a sample solution into the hollow core, which is typically 10's of mm in diameter, while maintaining the high optical transmission efficiency of the fibre. Confinement of both excitation light and sample solution within the core results in intense light-matter interactions over very long path-lengths (> 10cm).[1] As an optofluidic system for $^1O_2$ detection, HC-PCF offers two significant advantages: (i) the photosensitizer solution is subject to intense and homogeneous excitation along the entire length of the fibre core, resulting in efficient generation of $^1O_2$ from a sub-microlitre volume of photosensitizer; (ii) the $^1O_2$ luminescence is collected over the entire excitation path length and guided to the end of the fibre for detection. We have demonstrated the feasibility of directly detecting sub-picomole quantities of $^1O_2$ using this methodology.[2]

We will present new developments in the detection of $^1O_2$ produced by two-photon-induced photosensitization in HC-PCF. In conventional experiments, the extremely high photon intensity needed to achieve two-photon absorption is created by focusing a pulsed laser beam into a spot of about 1 μm in diameter. This miniscule excitation volume makes the detection of two-photon-induced $^1O_2$ generation extremely challenging. The unique optofluidic properties of HC-PCF offer a radically new approach to the study of two-photon photosenstization, since, within the core, two-photon excitation can be sustained over a path-length >10 cm.[3] When combined with highly sensitive detection of $^1O_2$ luminescence, this offers the prospect of a highly advantageous method for the *in vitro* screening and quantitative characterisation of two-photon photosensitizers, under mechanistically relevant conditions.

References

1. A. M. Cubillas, S. Unterkofler, T. G. Euser, B. J. Etzold, A. C. Jones, P. J. Sadler, P. Wasserscheid, P. S. Russell *Chem. Soc. Rev.* **2013**, 42, 8629-8648.
2. G. O. S. Williams, T. G. Euser, P. St. J. Russell, A. J. MacRobert, A. C. Jones *ChemPhotoChem* **2018**, 2, 616-621.
3. G. O. S. Williams, T. G. Euser, J. Arlt, P. S. J. Russell, A. C. Jones *ACS Photonics* **2014**, 1, 790-793.

> OC129. Oral Communication
Symposium PCHEM-6 Type-II photooxidation processes (Alec Greer)

TRACKING SINGLET OXYGEN IN HYBRID NANOMATERIALS AND PHOTOSENSITIZER DELIVERY SYSTEMS

Authors: Vladimir Kabanov[1], David J. Press[1], Sanjana Ghosh[2], Jonathan Lovell[2], Belinda Heyne[1]
Presenting Author: Vladimir Kabanov
1) Department of Chemistry, University of Calgary, Calgary, AB, Canada 2) Department of Biomedical Engineering, University at Buffalo, Buffalo, NY, USA

Production of singlet oxygen from light-activated drugs (photosensitizers) has been used to various degrees in photodynamic therapy to remove cancerous tumours and microbial pathogens. However, not surprisingly, singlet oxygen's highly reactive nature creates obstacles in studying and exploiting this elusive species' properties to their fullest extent.[1, 2] In the last decade, nanomaterials have emerged as a promising platform to establish control over the singlet oxygen production.[3] Nevertheless, application of nanomaterials in photodynamic therapy is still in its infancy and substantial knowledge on controlling this methodology is missing.[4] For instance, the degree of interaction between the singlet oxygen, produced from photosensitiser carrying nanovesicles, and the nanoparticles themselves has been largely overlooked in the field. Our recently published and current studies, working with silica and lipid-based photosensitiser carriers, have shown that the partition of singlet oxygen between the intra- and extra- vesicular space is a critical parameter which dictates "useful" singlet oxygen production efficiency from these hybrid nano-systems.[5]

Working with a range of BODIPY and xanthene photosensitizers covalently encapsulated in the SiO_2 matrix via oil-in-water synthesis methodology, we found these structures to release singlet oxygen into the external solvent environment with the efficiency ranging from 29% to 77% depending on the photosensitizer's distribution within the nanocarriers. The latter was also characterized and found to be dictated by the photosensitizer's hydrophobicity, which is in line with the nanoparticles' synthesis approach.

In the case of porphyrin-lipid based liposomes encapsulating chemotherapeutic drugs (doxorubicin or irinotecan),[6] singlet oxygen produced within the lipid membrane was found to partition unevenly between the internal, membrane and external liposomal spaces, while overal spending ~2/3 of its lifetime inside of the liposomes. This result is especially important to consider when discussing chemotherapeutic drugs' stability and their release kinetics dependence on the singlet oxygen production.[7]

My presentation will concentrate on discussing the method of tracking singlet oxygen partition between the different phases from hybrid nanomaterials in solution, and the implications of differing singlet oxygen release efficiency and partition when these nanomaterials are considered to be used in photodynamic studies.

References

[1] Ogilby, P. R. *Chem. Soc. Rev.* **2010**, 39, 3181-3209.
[2] Krasnovsky, A.A. Jr., In *Photodynamic therapy at the cellular level*. Research Signpost, Trivandrum-695 023, **2007**,17-62.
[3] Lucky, S. S., et al. *Chem. Rev.* **2015**, 115, 1990-2042.
[4] Martins Estevão, B., et al. *Phys. Chem. Chem. Phys.* **2015**, 17, 26804-26812.
[5] Kabanov, V., et al. *Chem. Commun*. **2018**, 54, 6320–6323.
[6] Carter, K. A., et al. *Nature Comm.* **2014**, 5:3546.
[7] Luo, D., et al. *Small*. **2016**, 12(22), 3039-3047.

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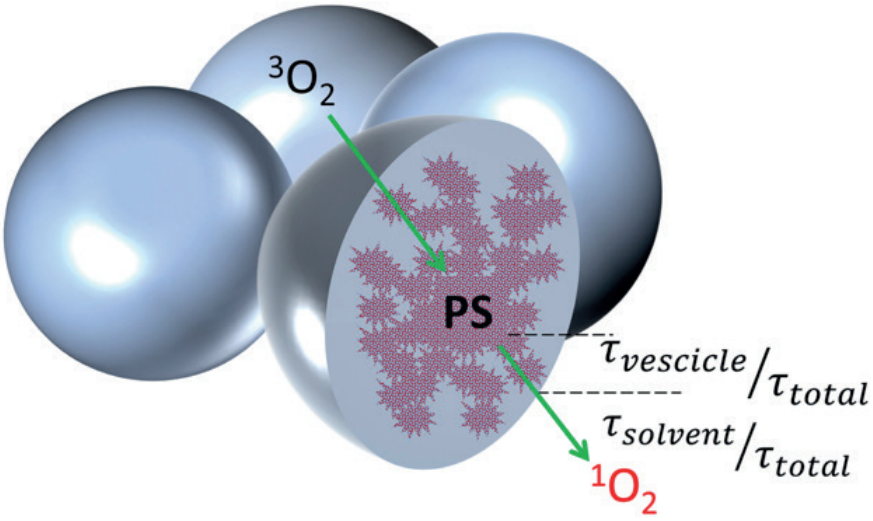
$^3O_2$
PS
$\tau_{vescicle}/\tau_{total}$
$\tau_{solvent}/\tau_{total}$
$^1O_2$

> P161. Poster

Symposium PCHEM-6 Type-II photooxidation processes (Alec Greer)

SINGLET OXYGEN REACTION WITH HISTIDINE DIPEPTIDE

Authors: Marisa H.G. Medeiros[1], Fernanda M. Prado[1], Paolo Di Mascio[1]
Presenting Author: Marisa H.G. Medeiros
1) Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo

Singlet Oxygen reacts selectively with a few amino acids residues in proteins, including cysteine, histidine, methionine, tryptophan, and tyrosine. The reported rates of reaction of singlet oxygen with histidine vary depending on the solvent and the pH of the solution. Photosensitized oxidation of histidine by singlet oxygen, yielding aspartic acid and urea via several intermediates has been described. In aqueous solutions, the reaction of singlet oxygen with histidine gives rise to peroxides (Di Mascio et al., 2019). Endogenous histidine-containing dipeptides such as carnosine (β-alanyl-L-histidine, CAR), homocarnosine (*gamma*-amino-butyryl-histidine) and anserine (*β*-alanyl-L-1-methylhistidine) have been recognized as detoxifying agents against reactive carbonyl species. Carnosine has the ability to react with singlet oxygen, and other reactive oxygen species. Carnosine reacts with singlet oxygen two to four times faster than histidine and twice faster than dipeptides histidine N-terminal residues. The reaction of carnosine with singlet oxygen has been described as one of the possible cardio-protection mechanisms of carnosine. However, the molecular mechanisms of these reactions are not completely described. Carnosine is abundantly expressed in the skeletal and cardiac muscles, as well as in other excitable cells. In skeletal muscle, β-alanine availability is a limiting factor for carnosine synthesis and β-alanine supplementation has been shown to consistently increase carnosine content in skeletal muscle. Previous studies have demonstrated that carnosine functions as an intracellular buffer in skeletal muscle. Other properties of carnosine include metal quenching, anti-glycation, and aldehyde detoxification. In this work, we investigated the reaction products of singlet molecular oxygen with carnosine using on-line reverse-phase high-performance liquid chromatography with electrospray ion-trap mass spectrometry detection. Carnosine (20 mM) was dissolved in D_2O containing 10 μM methylene blue and irradiated using light from a tungsten lamp (500 watts) filtered through a 360 nm cutoff filter. The positive mode ESI/MS analysis of the reaction showed products with protonated molecular ions $[M+H]^+$with m/z 247.10; 259,10; 275,09, 277,11. The products were isolated by reverse phase HPLC and characterized by ESI/MS/MS spectra analyses. Based on these results, the pathway of the reaction is proposed.

Supported by CEPID-Redoxoma (FAPESP: Proc. 2013/07937-8), NAP-Redoxoma (Pró-Reitoria de Pesquisa-USP: Proc. 2011.1.9352.1.8), CNPq (Proc. 301404/2016-0) and Universidade de São Paulo.

Reference

Di Mascio et al., Chem. Rev., 119 (3), 2043-2088, 2019.

> P162. Poster

Symposium PCHEM-6 Type-II photooxidation processes (Alec Greer)

SINGLET OXYGEN PHOTOSENSITIZATION BY CARBON NANODOTS PREPARED BY PULSED LASER SYNTHESIS

Authors: David García Fresnadillo[Depar], Sergio Ramírez Barroso[Depar], Antonio Ribeiro González[Depar], Mariona Cabero Piris[Insti], Pablo Díaz Núñez[Depar], Luis Bañares[Depar], Santi Nonell[Insti], Nazario Martín[Depar]
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Carbon nanoparticles such as carbon nanodots or graphene quantum dots (QDs) have recently attracted great interest because of their peculiar structure and optoelectronic properties, suitable for photobiological applications such as photodynamic therapy and biological imaging.[1] These carbon nanostructures may be core-doped or edge-decorated with a variety of heteroatoms and functional groups, allowing fine-tuning of their physicochemical properties and inner/surface functionality. Furthermore, carbon-based nanoparticles tend to show low toxicity and, therefore, could be ideal nanomaterials for theranostics, outperforming inorganic semiconductor QDs.[2]

In the present work, carbon nanodots have been prepared by one-step pulsed laser synthesis (nonfocused Nd-YAG, 10 Hz, 1064/532 nm, 3.5 W/cm^2) following a bottom-up approach starting from cheap organic aromatic precursors (*e.g.*, toluene), and inorganic compounds as heteroatom precursors for doping and functionalizing the carbon nanostructure. The carbon nanoparticles were structurally characterized by AFM, STEM microscopy combined with EELS and EDS, XPS and FTIR spectroscopies. Photophysical characterization was carried out by UV-VIS absorption spectrophotometry and emission spectroscopy (steady-state and time-resolved).

Generation of the carbon nanodots by pulsed laser synthesis follows a zero-order kinetics as determined from emission spectroscopy measurements of the reaction crude, pointing to a surface-catalyzed process which involves a change in C-atom hybridization from sp^2 to sp^3. Fluorescence emission is observed under UV-VIS excitation, with excitation wavelength-dependent emission maxima and emission quantum yields in the $0.01 < \Phi_{em} < 0.1$ range in 2-propanol. Emission decays require tri-exponential fitting with fluorescence lifetimes in the $1 < \tau_{em} < 20$ ns range. Singlet oxygen production quantum yields in the $0.02 < \Phi_{\Delta} < 1.00$ interval have been determined, depending on the presence of dopant heteroatoms and organic precursors used.

The structure, emission and photosensitization properties of a series of carbon nanodots have been characterized, showing moderate–high singlet oxygen photosensitizing ability in combination with moderate fluorescence in the VIS region, paving the way for the potential application of these nanomaterials in theranostics.

Financial support by the European Research Council ERC-320441-Chirallcarbon and the Ministerio de Economía y Competitividad (MINECO) of Spain (projects CTQ2014-52045-R CTQ2017-84327-P, CTQ2017-83531-R, CTQ2015-71896-REDT, CTQ2015-65033-P and CTQ2016-78454-C2-1-R) is acknowledged. The facilities provided by the Center for Ultrafast Lasers at Universidad Complutense de Madrid are gratefully acknowledged.

There are no conflicts to declare.

References

[1] (a) Y. Choi *et al*. *Adv. Funct. Mater*., **2014**, *24*, 5781-5789; (b) J. Ge *et al*. *Nature Commun*., **2014**, *5*, 4596; (c) K. Jiang *et al*. *Angew. Chem. Int. Ed*., **2015**, *54*, 5360-5363; (d) X. L. Liu *et al*. *Adv. Funct. Mater*., **2016**, *26*, 8694-8706; (e) A. Pramanik *et al*. *ACS Omega*, **2017**, *2*, 554-562.
[2] (a) K. Honda *et al*. *Nanotoxicology*, **2016**, *4*, 391-412; (b) T. B. Henry *et al*. *Environ. Pollut.*, **2018**, *232*, 191-199.

> P163. Poster

Symposium PCHEM-6 Type-II photooxidation processes (Alec Greer)

DETECTION OF PHOTOSENSITIZED SINGLET OXYGEN WITHIN SINGLE-RING PHOTONIC CRYSTAL FIBRES

Authors: Sergio Adan Bermudez[1], Anita C. Jones[1]
Presenting Author: Sergio Adan Bermudez
1) EaStCHEM School of Chemistry Joseph Black Building, The University of Edinburgh, Edinburgh, EH9 3FJ UK

The application of two-photon excitation (TPE) for the photosensitization of singlet oxygen in photodynamic therapy (PDT) increases the penetration depth and spatial selectivity, reducing the photodamage of healthy tissues. The difficulty of detecting photochemical reactions in the extremely small excitation volume of TPE presents a great barrier to the characterization of newly developed photosensitizers tailored for TPE. The direct detection of singlet oxygen ($^1O_2$) by its intrinsic phosphorescence at 1270 nm is very challenging, because of the extremely low quantum yield of this emission (10^{-5}-10^{-7}) and the low quantum efficiency of photodetectors operating at this wavelength.

Hollow-core photonic crystal fibres (HC-PCFs) are state-of-the-art optofluidic systems that have the ability to solve these problems. The potential of HC-PCF for applications in chemical sensing and photochemistry is beginning to be realised, including the ultra-sensitive detection of fluorescence with attomole sensitivity [1] and sensing luminescence of singlet oxygen at 1270 nm [2]. HC-PCF is particularly promising for the study of two-photon photosensitization since two-photon excitation can be sustained over a path-lengths of several centimetres within the fibre core [4] without significant transmission losses.

Single-ring anti-resonant reflection (ARR) fibre is a newly developed type of HC-PCF that significantly reduces the complexity and guidance losses in the core compared to previous generations [4]. We will report an investigation of the use of single-ring anti-resonant HC-PCF for the detection of two-photon photosensitized singlet oxygen, using a well-established fluorescent probe, singlet oxygen sensor green (SOSG) [5]. This novel approach exploits the long path-length over which TPE can be sustained and the co-confinement of both photosensitizer and fluorescent probe along this extended excitation path.

References

[1] G. O. S. Williams, T. G. Euser, P. S. J. Russell and A. C. Jones, Spectrofluorimetry with attomole sensitivity in photonic crystal fibres, *Methods Appl. Fluoresc.* 2013, 1, 015003.
[2] G. O. S. Williams, T. G. Euser, P. S. J. Russell, A. J. MacRobert and A. C. Jones, Highly Sensitive Luminescence Detection of Photosensitized Singlet Oxygen within Photonic Crystal Fibers, *ChemPhotoChem,* 2018, 2, 616-621.
[3] G. O. S. Williams, T. G. Euser, J. Arlt, P. S. J. Russell and A. C. Jones, Taking Two-Photon Excitation to Exceptional Path-Lengths in Photonic Crystal Fiber, ACS Photonics, 2014. 9, 790-793.
[4] A. M. Cubillas, X. Jiang, T. G. Euser, B. J. Etzold, P. Wasserscheid and P. S. J. Russell, Photochemistry in a soft-glass single-ring hollow-core photonic crystal fibre, Analyst, 2017, 142, 925-929.
[5] X. Ragàs, A. Jiménez-Banzo, D. Sánchez-García, X. Batlloria and S. Nonell, Singlet oxygen photosensitisation by the fluorescent probe Singlet Oxygen Sensor Green, Chem. Commun. 2009, 0, 2920-2922.

> P164. Poster

Symposium PCHEM-6 Type-II photooxidation processes (Alec Greer)

AMPHIPHILIC AABB-PHTHALOCYANINES AS NEW PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

Authors: Miguel Ángel Revuelta-Maza[1], Santi Nonell[2], Gema de la Torre[1,3], Tomás Torres[1,3,4]
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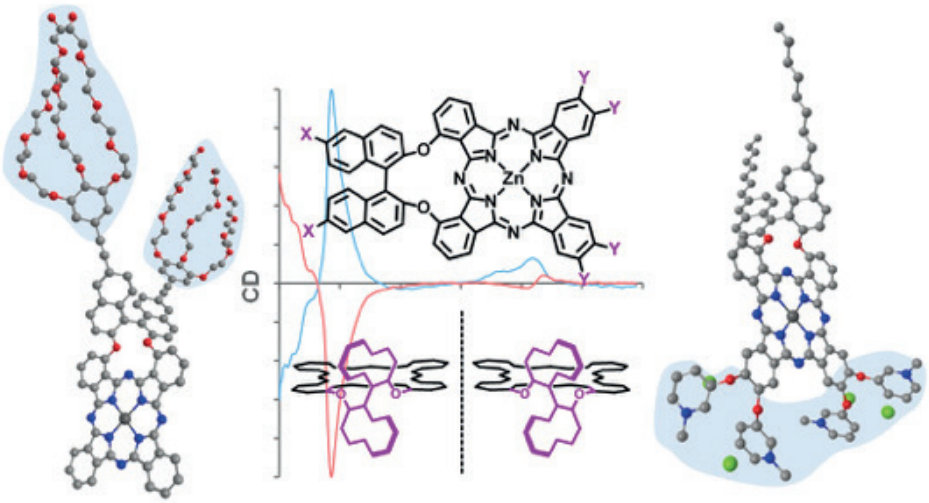
Phthalocyanines (Pcs) are utilized in various applications due to their outstanding photochemical characteristics. When the UV-Vis absorption maximum of the dye is moved to the near-IR region, their photosensitization properties can be utilized for photodynamic therapy (PDT), whenever they have long life-time triplet states, and adequate photosensitization of singlet oxygen.[1] To date, few works have been published about A_2B_2-Pcs due to their synthetic difficulties. Thus, two possible isomers, namely 'adjacently' (AABB-Pc, C_{2v} type) and 'oppositely' substituted (ABAB-Pc, D_{2h} type), are formed.[2] The best strategy to avoid the presence of one of the isomers is by using precursors with special features which do not allow the formation of the undesired isomer.[3] Herein, optically active AABB-type Zn(II) binaphthalo-phthalocyanines have been efficiently prepared via statistical condensation procedures using phthalonitriles as starting materials. Key to the selective preparation of the 'adjacent' isomers versus the 'opposite' ABAB ones is the use of (S)- or (R)-3,3\'-([1,1\'-binaphthalene]-2,2\'-diylbis(oxy))diphthalonitrile. The nature of the system can be changed either by introducing additional substituents onto the binaphthyl 6,6'-carbon atoms as well as onto the β positions of the other two isoindolic units, resulting in optically active highly functionalized Pcs. Amphiphilic properties will be gain by controlling the hydrophilic or hydrophobic character of the substituents, and lead to new systems for the construction of chiral organized nanoassemblies in aqueous media. When amphiphilic properties are gain by positive charges, Pc derivatives have proved to be efficient in the photoinactivation of S. aureus and E. coli.



> IL353. Invited Lecture
Symposium PCHEM-7 DNA damage (Thierry Douki)

LOOKING AT PHOTOSENSITIZED DAMAGE IN DNA AND BEYOND

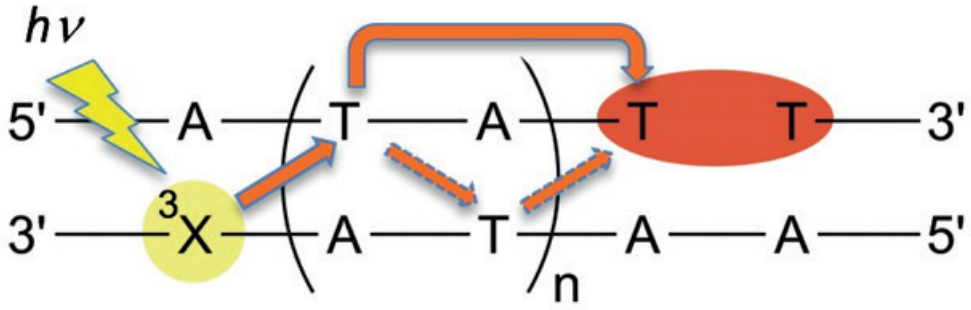
Authors: Lluís Blancafort[1], Alexander Voityuk[1], Christopher Grieco[2], Bern Kohler[2]
Presenting Author: Lluís Blancafort
1) Institut de Química Computacional i Catàlisi, Universitat de Girona 2) Department of Chemistry and Biochemistry, The Ohio State University

In this talk some of our recent results on triplet photosensitized damage in DNA are presented. We have modeled long-range triplet excitation energy transfer in DNA sequences of alternating adenine-thymine steps. This system has been studied experimentally,[1] and our computational model shows that the transfer consists of thermally induced hops through thymine bases on the same strand separated by an AT step.[2]

Beyond DNA, recent results on catechol will be presented, a molecule which is part of the melanin dihydroxyindol monomer. Here we have studied the effect of aggregation on the hydrogen elimination reaction (joint work with the Kohler group, Ohio State University).[3]

References

1. Antusch, L.; Gass, N.; Wagenknecht, H.-A. *Angew. Chem., Int. Ed.* **2017**, *56*, 1385.
2. Blancafort, L.; Voityuk, A. A. *Phys. Chem. Chem. Phys.* **2018**, *20*, 4997.
3. Grieco, C.; Kohl, F. R.; Zhang, Y.; Natarajan, S.; Blancafort, L.; Kohler, B. *Photochem. Photobiol.* **2019**, *95*, 163.



> IL355. Invited Lecture
Symposium PCHEM-7 DNA damage (Thierry Douki)

EFFECT OF SEQUENCE CONTEXT AND SENSITIZER ON DIRECT AND PHOTOSENSITIZED PHOTOPRODUCT FORMATION IN DNA.

Authors: John-Stephen Taylor[1], Chen Liu[1], Yanjing Wang[1]
Presenting Author: John-Stephen Taylor
1) Washington University in St. Louis

The lethal and mutagenic effects of UV light are the result of a complex interplay between factors that affect the formation of DNA photoproducts, their conversion to other photoproducts, their repair, and their bypass by polymerases. We will report on the development of DNA sequence libraries of the type NPyPyN for studying the effects of flanking sequence context on direct UVA/B/C formation of cis,syn cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts, and on photosensitized CPD formation. A strong quenching effect was observed for a 5'-flanking G on UV-induced formation of CPDs which can be attributed to electron transfer-mediated quenching of the excited state of the 5'-pyrimidine. We also found that the effect of flanking sequence on photosensitized CPD formation depended on the structure and properties of the photosensitizer. Photosensitized CPD formation is of particular interest given the recent discovery by Brash and coworkers of a chemiexcitation pathway to CPDs in melanocytes that is proposed to occur via triplet-triplet energy transfer to DNA from an excited state ketone produced from thermal decomposition of a high energy dioxetane. To gain further insight into this process, we have determined the triplet state energies and ability to photosensitize CPD formation of substituted aromatic ketones and aldehydes representative of products expected from the decomposition of dioxetanes of various endogenous metabolites.

> IL354. Invited Lecture
Symposium PCHEM-7 DNA damage (Thierry Douki)

FORMATION OF CYCLOBUTANE PYRIMIDINE DIMERS BY TRIPLET ENERGY TRANSFER

Authors: Miguel Miranda[1]
Presenting Author: Miguel Miranda
1) Universitat Politècnica de València

Formation of cyclobutane pyrimidine dimers (CPDs) upon UV-irradiation of DNA or its substructures may take place either upon direct excitation of the nucleobase or also through a photosensitized process. In this presentation, we focus on CPDs formation in model systems, triggered by triplet-triplet energy transfer from appropriate photosensitizers to the pyrimidine bases, with special emphasis on the following aspects: a) the role of triplet energies, b) the need for complexation, c) the possible involvement of delocalized triplet excited states, d) the long range migration of triplet excitation by a through-bond energy transfer mechanism, and e) the nature of the rate-controlling step (intermolecular energy transfer versus intramolecular excited state cyclodimerization) in bipyrimidine derivatives. The presented results contribute to a better understanding of the process, which still requires further investigation in spite of been known since decades.

> IL350. Invited Lecture
Symposium PCHEM-7 DNA damage (Thierry Douki)

UV-INDUCED GENOTOXIC PATHWAYS LEADING TO MELANOMA

Authors: Thierry Douki[1]
Presenting Author: Thierry Douki
1) Univ. Grenoble Alpes, CEA, CNRS, SyMMES UMR5819 Grenoble, France

Induction of DNA damage by sunlight is a key initiating event in the onset of skin cancer. Ultraviolet radiation (UV) represents the most efficient part of the solar spectrum mostly because of its ability to interact with biomolecules, in particular DNA. The mechanisms underlying the initiation step of basal and squamous cell carcinomas are now well understood. They result from the absorption of energetic UVB photons by DNA followed by formation of DNA photoproducts, in particular cyclobutane pyrimidine dimers (CPD).In contrast, the photochemical and photobiological events leading to the formation and accumulation of mutagenic DNA lesions is less well understood for melanoma. Evidence are growing that not only UVB but also less energetic but much more intense UVA is involved. The contribution of UVA is often explained by the photochemical properties of melanin. Indeed, while eumelanin is an efficient UV-absorbing photoprotective compound, pheomelanin exhibits some photosensitizing properties. As a result, melanocytes are more sensitive to UVA-induced oxidative stress than other cell types. One consequence of this process is the largest yield of oxidative DNA lesions in melanocytes than in keratinocytes that we observed, like others, in *in vitro* experiments. We were also interested in the UVA-induction of CPDs. Immediately after irradiation, the level of CPDs is similar in both melanocytes and keratinocytes. In the former cell type, CPDs are also produced in the dark after irradiation through an oxidative pathway. The resulting CPDs are yet rapidly removed by DNA repair. Altogether, one may expect that mutation spectra in melanoma would reflect the presence of oxidative lesions like 8-oxo-7,8-dihydroguanine or single stand breaks. Yet, recent next generation sequencing experiments have shown that the melanoma mutational signature is associated with the formation of pyrimidine dimers like carcinomas. The lack of significant contribution of oxidative lesions to mutagenesis may be explained by their efficient repair. Alternatively, a role for oxidative stress in melanoma may be associated with the decrease in repair capacities induced by UVA which is increasingly documented. Sunlight would thus have a synergistic effect on melanocytes with induction of pyrimidine dimers by UVB and decrease in their repair as the result of UVA exposure.

> IL352. Invited Lecture
Symposium PCHEM-7 DNA damage (Thierry Douki)

DNA DAMAGE IN HUMAN SKIN: IMPACT OF WAVELENGTH AND SKIN TYPE

Authors: Antony Young[1]
Presenting Author: Antony Young
1) King's College London, London, UK

Introduction
DNA photodamage initiates most skin cancers. Important factors are the location and type of damage. Black skin is much more susceptible to skin cancer than white skin. Previous studies that had compared the photoprotective properties of melanin against CPD had shown a relatively modest effect that did not explain the large differences between skin cancer incidence in black and white skins.

Methods
Healthy young volunteers of different skin types were irradiated on the buttocks with different spectral sources. Biopsies were taken at different times and processed for the detection of DNA photolesions. Melanin was quantified in some studies.

Results and Discussion
UVB (300nm) readily induces CPD and 6-4PP in the epidermis with a reduction of damage with epidermal depth. UVA1 (340-400nm) induces CPD, but not 6-4PP, with increasing damage with epidermal depth. This suggests the presence of a photosensitizer in the lower epidermis or back scatter from the dermis. In any case, the melanocyte and stem cell containing basal layer is particularly sensitive to UVA1-induced CPD. Interestingly, the repair UVA1-induced CPD in basal layer keratinocytes and melanocytes was negligible compared with lesions induced by UVB. This supports work that suggests that UVA damages the DNA repair machinery. Overall, these data suggest that UVA-1 may be more carcinogenic than previously thought.

Volunteers of skin phototypes VI (West African) and I/II (European) were irradiated with a range of doses of solar simulated radiation (SSR). The doses were adjusted to be erythemally equivalent. CPD were assessed in 3 epidermal zones – basal, middle and upper. There was no effect of epidermal zone on CPD in skin types I/II but there was a decrease in CPD with increasing epidermal depth in black skin that was related to melanin concentration. Melanin protection factors against CPD were calculated by comparing black and white skins. These were 8.0 for the overall epidermis, and 59.0, 16.5 and 5.0 for the basal, middle and upper epidermal zones respectively. The high protection by melanin in the basal layer concurs with the differences in skin cancer incidence in extreme phototypes.

Conclusions
These studies show that assessments over the whole epidermis may give misleading results and suggest that epidermal location is a critical factor in skin cancer risk assessment of human skin.

Acknowledgements
The research was funded by the UK Medical Research Council (MRC) and Walgreens Boots Alliance Inc.

References
1. A Tewari, RP Sarkany, AR Young (2102). UVA1 induces cyclobutane pyrimidine dimers but not 6-4 photoproducts in human skin in vivo. J Invest Dermatol. 132:394-400.
2. D Fajuyigbe, SM Lwin, BL Diffey, R Baker, DJ. Tobin, RPE Sarkany, AR Young (2018). Melanin distribution in human epidermis affords localized protection against DNA photodamage and concurs with skin cancer incidence difference in extreme phototypes FASEB J. 32: 3700–3706.

> OC130. Oral Communication
Symposium PCHEM-7 DNA damage (Thierry Douki)

BIPHOTONIC CHEMISTRY OF PYRIMIDINE DERIVATIVES

Authors: Ofelia R. Alzueta[1], M. Consuelo Cuquerella[1], Miguel A. Miranda[1]
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1) Av. Los Naranjos s/n 46022, Valencia, Spain

The DNA biomacromolecule can suffer structural modifications leading to lesions and mutations due to external and internal factors, as exposure to ultraviolet solar radiation that has been unequivocally demonstrated to be involved in skin cancer. In this context cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4 PPs) represent the 75% and 25%, respectively, of the total photoinduced dimeric lesions.[1] Despite the intensive efforts made to elucidate the photoreaction mechanism of DNA bases, further research is still needed in the field. According to the generally accepted paradigm, 6-4 PPs formation occurs from an excited singlet state upon direct UV irradiation, whereas cyclobutanes can arise from both the lower singlet and triplet excited states. In a previous work, it was demonstrated that part of the processes attributed to singlet excited states could occur from upper triplet excited states. This concept was proven with 5-*tert*-butyl uracil; when its T_2 is populated by means of high energy laser pulses, a Norrish-Yang reaction takes place giving rise to the corresponding pyrimidone.[3]Now, we propose an alternative intermolecular approach. Thus, 2'-methoxyacetophenone (2M) has been used as a photosensitiser due to its capability to be selectively excited at wavelengths longer than 300 nm and taking advantage of the lack of oxetanes formation as a result of a Paterno-Büchi reaction, observed with other photosensitisers such as benzophenone.[4] In this approach the population of thymine T_2 (np*) is achieved by a biphotonic excitation of its T_1 populated as a result of an energy transfer from the photosensitiser. Two pyrimidine derivatives were selected to test this methodology in two different reactions: a) 5-*tert*-butyl uracil ester (1) to accomplish Norrish-Yang photocyclisation (3) and b) uracil ester (2) to obtain the hydrated photoproduct (4). Both are assumed to be singlet state reactions. Biphotonic irradiations of highly concentrated solutions of 1 or 2 in the presence of 2M were performed and then analysed by UPLC coupled with tandem mass spectrometry. Several controls were undertaken to rule out a direct irradiation, as for example biphotonic irradiations of solutions without photosensitiser or monophotonic irradiation under otherwise the same conditions. The assignment was unambiguously confirmed by independent synthesis of the photoproducts. Moreover, a comparison of the pyrimidone photoproduct formation through the 5-*tert*-butyl uracil ester photosensitisation with three different photosensitisers (benzophenone, fenofibrate and 2M), resulted in a higher effectiveness in the case of 2M. This paves the way to 2M application as an efficient photosensitiser in biphotonic approaches to study molecular processes in pyrimidine derivatives

References

1. Cuquerella, M. C.; Lhiaubet-Vallet, V.; Bosca, F.; Miranda, M. A. *Chem. Sci.* **2011,** *2* (7), 1219-1232.
2. Vendrell-Criado, V.; Rodríguez-Muñiz, G. M.; Yamaji, M.; Lhiaubet-Vallet, V.; Cuquerella, M. C.; Miranda, M. A. *J. Am. Chem. Soc.* **2013,** *135* (44), 16714-16719.
3. Liu, L.; Pilles, B. M.; Reiner, A.M.; Gontcharov, J.; Bucher, D.; Zinth, W. *ChemPhysChem* **2015**, *16*, 3483

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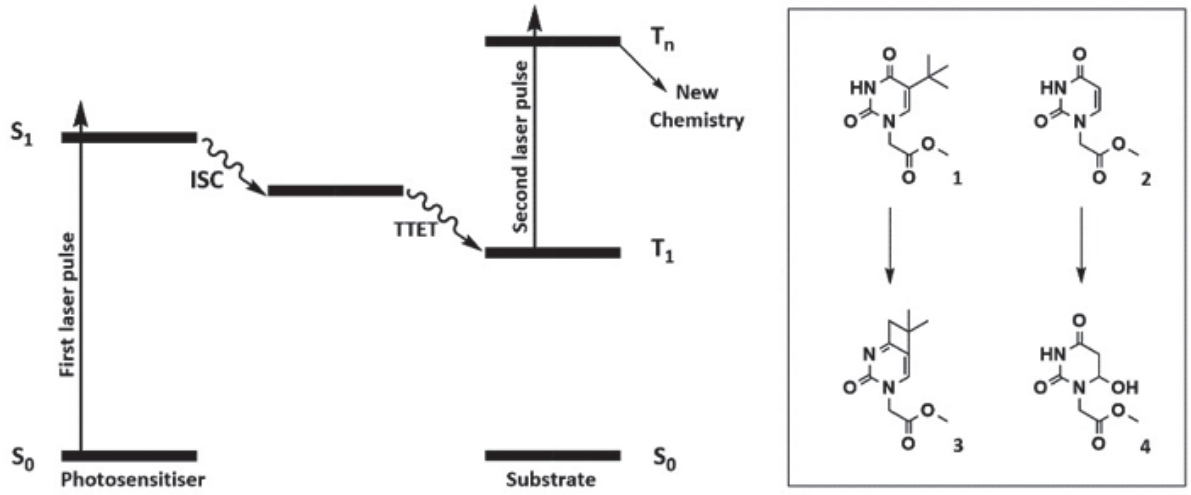
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> OC131. Oral Communication
Symposium PCHEM-7 DNA damage (Thierry Douki)

PHOTOKINETICS AND PHOTOSTABILISATION OF ETHYLHEXYL METHOXYCINNAMATE

Authors: Bernd Herzog[1], Volker Settels[2], Lola Amoros-Galicia[1], Myriam Sohn[1], Giesinger Jochen[1]
Presenting Author: Bernd Herzog
1) BASF Grenzach GmbH 2) BASF SE

Ethylhexyl methoxycinnamate (EHMC) is an oily UV-absorber used in sunscreens for the photoprotection of human skin and represents one of the most employed UVB absorbers for that application. We studied the photodegradation and photostabilisation of this molecule. By adding a non-absorbing oil, the EHMC concentration in the oil phase of water-in-oil emulsions was varied, while keeping the overall EHMC concentration in the emulsion constant. A second-order rate law of the photokinetics was observed, in line with the [2+2]-cycloaddition reaction mechanism known for this UV absorber. The second-order rate constant decreased with higher overall EHMC concentration, what can be explained by the fact, that less photons are absorbed per molecule at higher overall concentration of the UV absorber. On the other hand, the rate constant increased with decreasing polarity of the surrounding oil. Since the molar fraction of the trans-isomer of EHMC is augmented at lower polarity, more photons are absorbed in this case, as the absorption strength of the trans-isomer is significantly higher compared to that of the cis-isomer. In conclusion, a high polarity of the oil phase and a high concentration of EHMC are advantageous for the photostability of this compound. On the other hand, the photostabilty of EHMC can be also increased by adding certain UV-absorbers, such as octocrylene (OCR) or bis-ethylhexyloxy methoxyphenyl triazine (BEMT). It is shown experimentally that in both cases the stabilization is caused by a combination of a quenching and a filter effect due to the presence of the added UV-absorber. The quenching mechanism is supported with DFT calculations yielding the energy levels of excited singlet and triplet states of the three molecules.

> P165. Poster

Symposium PCHEM-7 DNA damage (Thierry Douki)

HOW CLUSTERED (CYCLOPURINES/GOXO) LESIONS "CHANGE" DNA DOUBLE HELIX. THE THEORETICAL APPROACH OF CHARGE TRANSFER AND STRUCTURE.

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The nucleus *ds*-DNA contained 3.2x10^9 base pairs and has been continuously exposed to different harmful factors like ionisation radiation, carcinogens, radicals. ect. Until now more than 80 different kinds of DNA damage were identified **[1]**. Reactions of OH● with nucleotides, may lead to the generation of radicals in the base and 2-deoxyribose moieties. The prerequisite for the formation of (5\'*R*) and (5\'*S*) 5\',8-cyclo-2\'-deoxyAdenosnine (**cdA**) is the one of the 5\'-CH_2 proton abstraction with subsequent C5\›-C8 cyclisation **[2]**. Majority of DNA damage are removed from genome by BER or NER. This two systems perform well in the case of simply lesions like AP sites or 8-oxo-7,8-dihydro-2'-deoxyguanosine (**G^{oxo}**), which have been recognised as most frequent damage. In contrast to this, the clustered lesion contained tandem lesion cdA and **dG^{oxo}** are the serious challenge for the cell repair system **[3]**. In favourable conditions, once *ds*-DNA has been oxidized, the formed radical cation "hole" can hop reversibly through the double-helix until it is trapped, usually by the reaction of the formed guanine radical cation (G$^{•+}$) with H_2O **[4]**. Depending on the nature of the G:::C base pair radical, two reaction paths are possible with or without the addition of a water molecule [**4,5**]. Due to the distortion, forced by both cdA diastereomers, of the spatial geometry and π stacking interaction in *ds*-DNA in this work the influence of **cdA** and **G^{oxo}** on the radical cation distribution and oxidation process was examined. For this studies the short oligonucleotides contained the presented below scheme of lesion distribution has been taken under theoretical consideration. The spin distribution of vertical cation as well as HOMO of neutral form elucidated the influence of **cdA** on the double helix electronic properties.

The sheme of **cdA** and **G^{oxo}** distribution in *ds*-DNA was as follow:

A) Native ds-DNA: **ApN**-d[AG**A**GGA]*d[TCCTCT]; **ApN-3\'G^{oxo}**-d[AG^{oxo}**A**GGA]*d[TCCTCT]; **ApN-5\'G^{oxo}**-d[AG**A**G^{oxo}GA]*d[TCCTCT]; **ApN-5\'3\'G^{oxo}**-d[AG^{oxo}**A**G^{oxo}GA]*d[TCCTCT]

B) ds-DNA contained 5\›*S* or 5\›*R* cdA moiety: **ApR/S**-d[AG**cA**GGA]*d[TCCTCT]; **ApR/S-3\'G^{oxo}**-d[AG^{oxo}**cA**GGA]*d[TCCTCT]; **ApR/S-5\'G^{oxo}**-d[AG**cA**G^{oxo}GA]*d[TCCTCT]; **ApR/S-5\'3\'G^{oxo}**-d[AG^{oxo}**cA**G^{oxo}G-A]*d[TCCTCT]

The theoretical approach has been performed on M06-2x/D95*/UFF level of theory in aqueous phase [6,7,8].

Conclusion

The appearing of both cdA in the *ds*-DNA structure leads to double helix distortion. The cdA presence in *ds*-DNA change the vertical ionisation potential of discussed systems the "global" VIP of *ds*-DNA contained G^{oxo} was as follow in [eV]: native *ds*-DNA: ApN(6.12)**>**ApN-5\'G^{oxo}(5.87)**>**ApN-3\'G^{oxo}(5.79)**>**ApN-5\'3\'G^{oxo}(5.78); (5\'*S*)cdA-DNA: ApS(6.20)**>**ApS-3\'5\'G^{oxo}(5.91)**>**ApS-3\'G^{oxo}(5.90)**>**ApS-5\'G^{oxo}(5.86); (5\'*R*)cdA-DNA: ApR(6.13)**>**ApR-5\'G^{oxo}(6.04)**>**ApR-3\'G^{oxo}(5.89)**>**ApR-5\'3\'G^{oxo}(5.88). Therefore due to above the upset of hole migration process can be expected.

Acknowledgments

This study was supported by grant **Nr 2016/23/B/NZ7/03367** and ACC Cyfronet AGH.

Reference

1)*FASEB J.*, 2003, 17, 1195, 2) *Acta Bioch. Pol.* 2009, 56, 655, 3) *Curr. Med. Chem.,* 2018, 25, 1, 4) *J. Am. Chem. Soc.* 2014, *136*, 5956, 5) *Chem. Rev.* 2010, *110*, 1642, 6) *J. Phys. Chem. A,* 2005, *109*, 5656, 8) *Gaussian 09, Revision A.02.*

> P166. Poster

Symposium PCHEM-7 DNA damage (Thierry Douki)

IDENTIFICATION AND CHARACTERIZATION OF NEW PHOTOADDUCTS FROM UVA MEDIATED REACTION BETWEEN N-NITROSOPROLINE AND DNA

Authors: Sakae Arimoto-Kobayashi[1], Shuhei Aoyama[1], Chiharu Asahi[1], Kayoko Sano[1], Tsutomu Hatano[1], Sachiko Kimura[2]
Presenting Author: Sakae Arimoto-Kobayashi
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N-Nitrosoproline (NPRO) is known to form endogenously from sodium nitrite and the amino acid proline, and is thought to be non-mutagenic and non-carcinogenic. However, earlier studies in our laboratory showed that irradiated NPRO can be converted directly to a mutagenic compound upon UVA irradiation. We investigated the mutagenic spectrum of NPRO on M13mp2 DNA with UVA irradiation,and the most frequent mutation was comprisedGC-to-CG and GC-to-TA, anda hot spot AT to GC. From UVA-irradiated solutions of NPRO and 2'-deoxyguanosine (dG), we isolated and identified new guanine adducts, (*R*)- and (*S*)-8-(2-pyrrolidyl)-2'-deoxyguanosine (G1 and G2, respectively), in addition to 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), 2'-deoxyxanthosine and 2'-deoxyoxanosine. We also found new adenine adducts, (*R*)-, (*S*)-2-(2-pyrrolidyl)-2'-deoxyadenosine (P1 & P2), (*R*)-,(S)-8-(2-pyrrolidyl)-2'-deoxyadenosine (A1 & A2)and deoxyinosine (dI) from the mixture of dA and NPRO irradiated with UVA. These adducts might be responsible for the mutation in phage M13mp2 treated with NPRO and UVA. Experiments using monochromatic UVA in the range 300-400 nm were performed. The highest yields of both G1 and G2 were found to occur at 340 nm, absorption maximum of NPRO. NPRO has no absorption at 400 nm, and no G1 nor G2 was detected from the sample of NPRO and dG irradiated at 400 nm. Wavelength-dependent formation of A1 & A2 also coincided with the absorption curve of NPRO, suggesting that sensitization of NPRO by UVA triggers the formation of G1, G2, A1 and A2.

We\›ve investigated the formation of photoproducts (A1, A2, P1, P2, G1, G2 and 8-oxodG) in the UVA irradiated DNA with NPRO. Samples prepared from DNA irradiated withUVA with NPRO at pH 7.0 under the air-condition were analyzes with LC-MSMS. Our investigation using calf thymus DNA showed that G1 and/or G2, probably both, A1 and/or A2, P1, P2 and 8-oxodG could be produced by UVA irradiation of DNA with NPRO as well. Formation of A1/A2, P1 and P2 were dependent on irradiation time and NPRO concentration. Production of P1/P2 and A1/A2 from irradiated NPRO with DNA increased under N_2saturation compared to those under air saturation, suggest that a Type-I mechanism is involved in the photoproduction of P1/P2 and A1/A2. ROS generated during photo-reaction may also act to destroy the activated form of irradiated NPRO to reduce the formation of photoadducts. Production of G1/G2 and 8-oxodG from irradiated NPRO with DNA slightly decreased under N_2compared to those under air, suggest that ROS or nitrite created via photo-reaction might beresponsible in part.

These photoproducts from UVA-irradiated NPRO with DNA may be related to mutagenic responses that result in DNA exposed to NPRO with UVA irradiation. We propose that endogenous NPRO might play a role in the photogenotoxicity of sunlight.

This work was supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education (15K00556 to S.A-K.).

> P167. Poster

Symposium PCHEM-7 DNA damage (Thierry Douki)

SPECTROSCOPIC STUDIES OF DNA ADDUCTS ARISING FROM POLYUNSATURATED FATTY ACID OXIDATION.

Authors: Paloma Lizondo-Aranda[1], Thomas Gustavsson[2], Miguel Ángel Miranda[1], Virginie Lhiaubet-Vallet[1]
Presenting Author:Paloma Lizondo-Aranda
1) Instituto Universitario Mixto de Tecnología Química (UPV-CSIC), Universitat Politècnica de València, Avda de los Naranjos, s/n., 46022, Valencia, Spain 2) Laboratoire Interactions, Dynamiques et Lasers (LIDYL) - CEA/Saclay, 91191 Gif-sur-Yvette, France

Lipid peroxidation is a biochemical process that is constantly occurring in our body due to the effect of diverse reactive oxygen species (ROS). These species attack the polyunsaturated fatty acids of the membrane triggering a self-propagating chain reaction, and provoking degradation of the membrane. From a chemical point of view, this results in the formation of reactive aldehydes such as malondialdehyde (MDA), which can interact with DNA bases inducing lesions known as etheno adducts.

In recent investigations, our group showed that some DNA lesions act as effective endogenous photosensitizers that might induce generation of a clustered damage in DNA. [1]

In this context, the etheno derivatives are of interest as they present an extended p-conjugated system that should result in a red-shifted absorption by respect with the canonical nucleobases. In order to study if these compounds exhibit the capacity to act as an efficient DNA photosensitizer, spectroscopic studies have been carried out.

TheedA and m1dG lesions present fluorescence emission in the visible region with lifetimes in the nanosecond timescale. By contrast, edG and edC appeared to suffer an ultrafast deactivation. Thus, upconversion fluorescence experiments were performed to get more insight into their emission properties. Finally, transient absorption spectroscopy was also carried out to establish the generation of further transient species.

Acknowledgements

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References

[1] V. Vendrell-Criado, G. M. Rodríguez-Muñiz, M.C. Cuquerella, V. Lhiaubet-Vallet and M. A. Miranda, Angew. Chem. Int. Ed. 2013, 52, 6476 –6479. I. Aparici-Espert, G. Garcia-Lainez, I. Andreu, Miguel A. Miranda and Virginie Lhiaubet-Vallet, ACS Chem. Bio. 2018, 13, 542−547.

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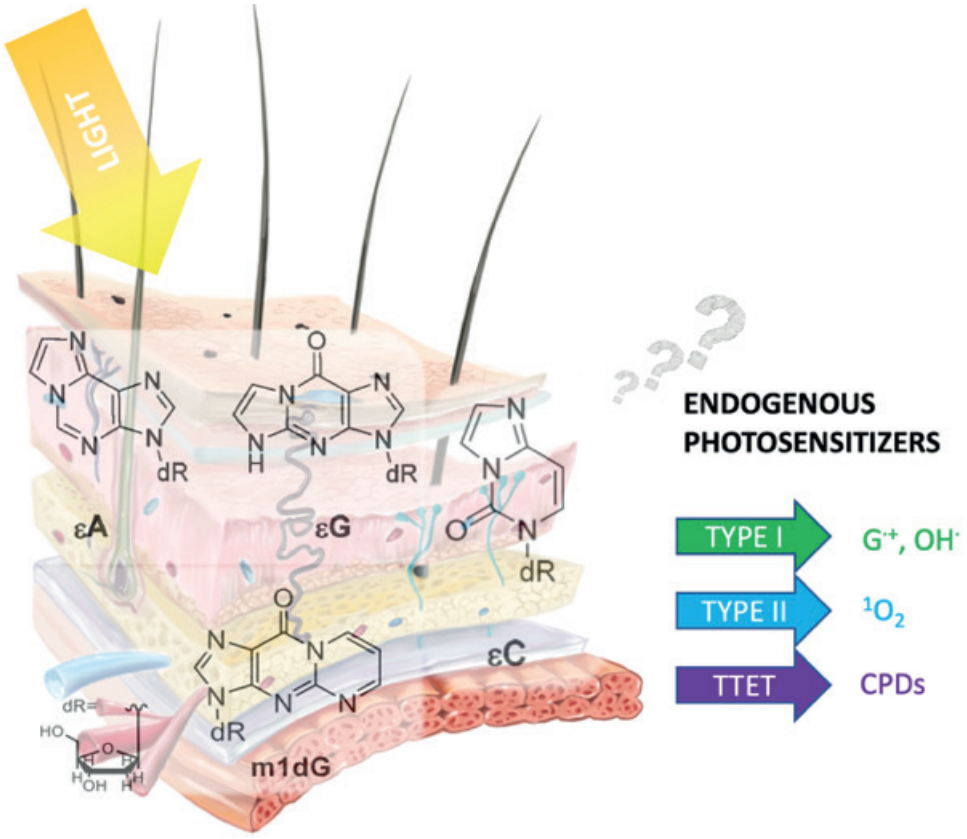

LIGHT
εA
εG
εC
m1dG
dR
ENDOGENOUS
PHOTOSENSITIZERS
TYPE I
G·+, OH·
TYPE II
1O2
TTET
CPDs

> IL359. Invited Lecture
Symposium PCHEM-8 Artificial Photosynthesis (Ana Moore)

ENGINEERING QUANTUM COHERENCE IN BIO-INSPIRED SYSTEMS FOR EFFICIENT SOLAR-ENERGY CONVERSION

Authors: Elisabet Romero[1]
Presenting Author:Elisabet Romero
1) The Institute of Chemical Research of Catalonia (ICIQ)

Photosynthesis holds the key to the efficient use of solar energy by humans using abundant and renewable materials. At the heart of Photosynthesis, the pigment-protein complex photosystem II reaction center (PSII RC), performs charge separation with near unity quantum efficiency despite its highly disordered energy landscape, and thus converts sunlight to electrochemical energy.

To achieve this amazing feat, the PSII RC exploits *The Quantum Design Principles of Photosynthetic Charge Separation*[1-2], complementary and interrelated solutions to ensure rapid forward and irreversible transfer of energy and electrons within a disordered and fluctuating environment. Therefore, these principles provide a guide for the rational design and construction of systems able to transfer energy and electrons with high efficiency and in the right direction. Here I will present these principles and focus on the key parameters that will lead to the implementation of quantum coherence in bio-inspired systems with the potential to perform efficient energy and electron transfer, with the final goal of achieving the cost-effective conversion of solar energy to fuel.

References

1. Romero, E., Augulis, R., Novoderezhkin, V. I., Ferretti, M., Thieme, J., Zigmantas, D. & van Grondelle, R. Quantum coherence in photosynthesis for efficient solar-energy conversion. *Nat. Phys.* **10**, 676-682 (2014).
2. Romero, E., Novoderezhkin, V. I. & van Grondelle, R. Quantum design of photosynthesis for bio-inspired solar-energy conversion. *Nature* **543**, 355-365 (2017).

> IL356. Invited Lecture
Symposium PCHEM-8 Artificial Photosynthesis (Ana Moore)

CAROTENOIDS CRYSTALLOIDS INSIDE CHROMOPLASTS EXHIBIT SINGLET EXCITON FISSION

Authors: Manuel Llansola-Portoles[1], Anja Krieger-Liszkay[1], Annamaria Quaranta[1], Simona Streckaite[1], Cristian Ilioaia[1], Kipras Redeckas[2], Mikas Vengris[2], Andrew Pascal[1], Alison Telfer[4], Leonas Valkunas[3], Bruno Robert[1]
Presenting Author: Manuel Llansola-Portoles
1) Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ Paris-Sud, Université Paris-Saclay, 91198, Gif-sur-Yvette cedex, France 2) Quantum Electronics Department, Faculty of Physics, Vilnius University, Saulėtekio Ave. 10, LT-10223, Vilnius, Lithuania 3) Molecular Compounds Physics Department, Center for Physical Sciences and Technology, Sauletekio Ave. 3, LT-10257, Vilnius, Lithuania 4) Faculty of Natural Sciences, Department of Life Sciences, Imperial College London

We have studied the structure and photochemistry of carotenoid in chromoplast from tomatoes and daffodil petals. These two chromoplasts contain crystalloids of lycopene and lutein that give the red color to the tomatoes and yellow color to the daffodil petals, respectively. Transient absorption studies conducted on lycopene and lutein crystalloids inside chromoplasts reveal the appearance of a long-lived (µs) excited state. The detection of the carotenoid triplet signature in resonance Raman allowed the identification of this long-lived specie as lycopene and lutein triplet, respectively. These triplet states must be generated by singlet exciton fission. This is the first time the singlet fission process has ever been shown to occur in a biological material. The biological function behind this singlet fission remains unclear, but we tentatively propose that it may be a photoprotective process, eventually inherited from photoprotection during chloroplast/chromoplast maturation or senescence.

Reference

Llansola-Portoles, M. J., K. Redeckas, S. Streckaité, C. Ilioaia, A. A. Pascal, A. Telfer, M. Vengris, L. Valkunas and B. Robert (2018). “Lycopene crystalloids exhibit singlet exciton fission in tomatoes.” Physical Chemistry Chemical Physics 20(13): 8640 - 8646.

> IL360. Invited Lecture
Symposium PCHEM-8 Artificial Photosynthesis (Ana Moore)

MOLECULAR MODULES FOR ARTIFICIAL PHOTOSYNTHESIS

Authors: Ally Aukauloo[1,2], Winfried Leibl[2], Thanassis Coutsolelos[3]
Presenting Author: Ally Aukauloo
1) University Paris Saclay 2) CEA Saclay 3) University of Heraklion

Understanding the functioning of Photosystem II, the enzyme that contains all the necessary chemical information to use light to oxidize water through a four-electron and four-proton process is a prerequisite to the development of artificial photosynthesis. The role of the Tyr_Z/His_{190} pair in PSII has for long been restricted to electron relay involving short range proton rocking mechanism between the oxidized tyrosine and the histidine. Recent X-ray and theoretical studies on PSII promote this role to a proton exit channel conveying proton from the oxygen evolving catalyst to the lumenal side of thylakoid.

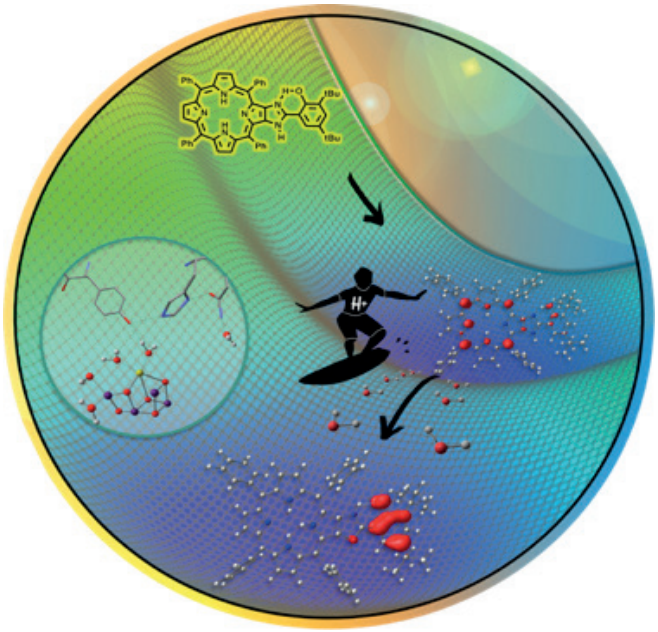
1) I will discuss on a model of Tyr_Z/His_{190} where we found that water molecules are crucial to unlock the electron transfer process from the photoxidized porphyrin and the phenol/imidazole pair.

2) I will present our study on light triggered two electrons charge accumulation process.

3) I will also discuss on new catalysts for the selective reduction of CO2 to CO.

References

1. Angew. Chem. Int. Ed. 2018, 57, 9013 –9017
2. Angew. Chem. Int. Ed. 2017, 56, 15936 –15940
3. Angew. Chem. Int. Ed, 2019. https://doi.org/10.1002/anie.201814339



> IL362. Invited Lecture
Symposium PCHEM-8 Artificial Photosynthesis (Ana Moore)

ARTIFICIAL PHOTOSYNTHESIS AT LOW PH

Authors: Lei Wang[1], Renato Sampaio[1], Javier Concepcion[1]
Presenting Author: Javier Concepcion
1) Brookhaven National Laboratory

Dye-sensitized photoelectrosynthesis cells (DSPEC) are one of many approaches to artificial photosynthesis and they are arguably the most commonly used approach for molecular components. In a basic configuration, a DSPEC is composed of a transparent conductive oxide (TCO) glass, a semiconductor metal-oxide film, and a combination of chromophores and catalysts. Traditionally, water oxidation takes place at the photoanode while H^+ or CO_2 reduction are conducted at a dark electrode or at a photocathode. In addition to the individual requirements for each component, there are many aspects related to how these components are assembled and how their interactions allow for proper function. Water oxidation at the photoanode is the result of many competing processes over a wide range of timescales from femtoseconds-picoseconds to seconds and it is the most challenging step. Because of all this functional complexity and the complexity associated with preparing and assembling the individual components, the photoanode of a DSPEC is basically a “black box” where scientists introduce these components and evaluate the outcome in terms of photocurrents, faradaic efficiencies/quantum yields for oxygen generation, and durability. Fundamental studies have been lacking and many important questions remain unanswered: what is the best material for the metal-oxide film? Recent studies suggest that maybe core-shell metal-oxide materials are the answer. What is the optimal chromophore (**C**)? One with the right absorption profile and the right thermodynamics for excited state electron injection and catalyst activation. What is the most favorable pH that will enable fast catalysis without sacrificing injection yields? What is the right pH to ensure long-term stability of the anchoring groups while allowing fast proton transport to the cathode? What is the optimal chromophore-catalyst distance to achieve the best balance between forward/backward electron transfer and catalysis? We have developed a new anchoring strategy that is allowing us to answer many of these questions. It is based on self-assembled bilayers (**SAB**) enabled by non-covalent interactions between long alkyl chains on the water oxidation catalyst (**Cat**) and a self-assembled monolayer (**SAM**) on the metal oxide surface (**SAM@MO$_x$**). With this strategy, we have been able to anchor catalysts to planar electrodes (**Cat-SAB@MO$_x$**) at various distances and study how heterogeneous electron transfer rates are affected. More importantly, we have been able to “synthesize” **C-Cat** assemblies on high surface area mesoporous electrodes (**Cat-SAB-C@MO$_x$**) and use them as photoanodes in DSPEC, Figure 1. The **SAB** strategy have made possible the assembly of various **C-Cat** combinations at various **C-Cat** distances, enabling us to tackle many of the questions presented above. **DSPEC studies with core-shell structures using a *nano*-ITO core and a ~ 4 nm TiO$_2$ shell in Cat-SAB-C@MO$_x$ photoanodes are consistently displaying photocurrents larger than 2 mA/cm^2, even at pH 1!** They also display stabilities orders of magnitude higher than previously reported DSPEC using other strategies.

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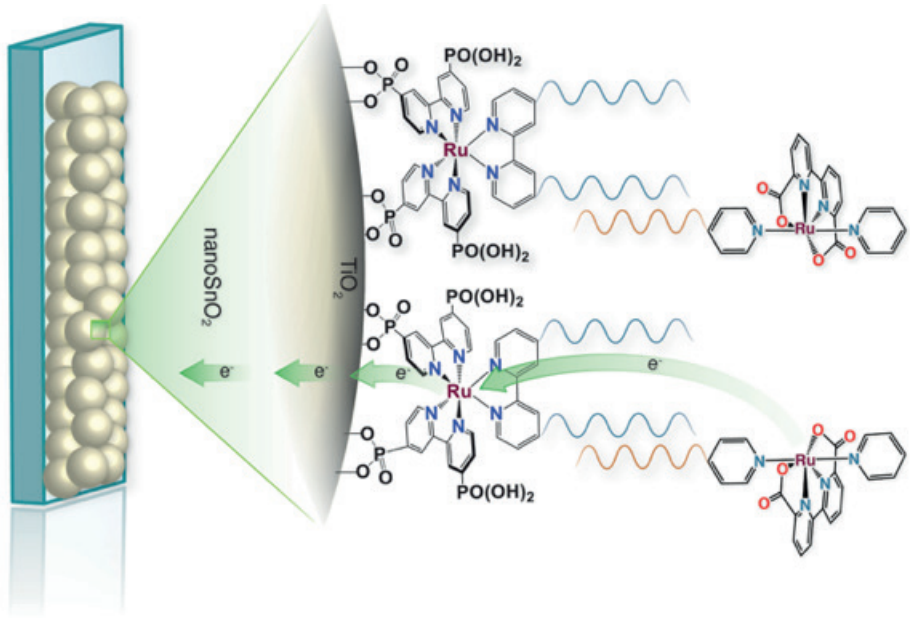
nanoSnO2
TiO2
e-
PO(OH)2
Ru

> IL357. Invited Lecture
Symposium PCHEM-8 Artificial Photosynthesis (Ana Moore)

MULTIPLE PROTON TRANSFERS COUPLED TO A SINGLE ELECTRON TRANSFER IN ARTIFICIAL PHOTOSYNTHETIC CONSTRUCTS

Authors: Ana L. Moore[1], Emmanuel Odella[1], S. Jimnena Moora[1], Brian Wadsworth[1], Gary F. Moore[1], Devens Gust[1], Thomas A. Moore[1]
Presenting Author: Ana L. Moore
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In photosystem II, tyrosine Z (Y_z) functions as a redox relay between the photo-oxidized primary donor ($P680^{\bullet+}$) and the oxygen-evolving complex (OEC), where water oxidation takes place. The oxidation of Y_z by $P680^{\bullet+}$ likely occurs with the transfer of the proton to a hydrogen-bonded histidine (His190).[1] Benzimidazole-phenol (BIP) and several of its derivatives have been synthesized to mimic the behavior of the Y_z-His190 pair. The phenol is a model of Y_z, and the benzimidazole is a model of His190. With a simple BIP, proton transfer from the phenol to the imidazole takes place upon oxidation the phenol; this is known as an electron-proton transfer (EPT) process. A one-electron two-proton transfer, known as an E2PT process, has been shown to take place in amino-substituted BIPs upon the electrochemical oxidation of the phenol. In this case, a decrease in the redox potential of the phenoxyl radical/phenol couple by ~300 mV was observed.[2]Theoretical calculations predicted that substituents with reduced pK_a's, such as substituted imines attached to BIP, would still undergo an E2PT process while maintaining the considerably higher potential for the phenoxyl radical/phenol couple necessary for a redox relay involved in the oxidation of the OEC. Thus, as alternative models of the Y_z-His190 pair, BIP with imine substituents were synthesized and results indicate that the phenol oxidation in these derivatives occurs at ~300 mV higher potential than in the amino-BIPs. Protonation of the benzimidazole, indicating an EPT process and protonation of the imine, indicating an E2PT process can be unambiguously detected by infrared spectroelectrochemistry (IRSEC) upon oxidation of the phenol. IRSEC results demonstrate that an E2PT process takes place with sufficiently strong electron-donating groups at the *para*-position of the N-phenylimine group (e.g.,$-OCH_3$ substitution). But when the imine basicity is reduced (e.g., with −CN substitution of the N-phenylimine group), an EPT product is dominant. Thus, the mechanism and consequently the extent of proton translocation along the H-bond network, either ~1.6 Å or ~6.4 Å, can be controlled through structural design.[3]One of the aims of this study is to determine how many proton transfers can be associated with one oxidation event and the construction of proton wires where proton transport across lipid bilayers to generate proton-motive force is accomplished in conjunction with redox reactions. (Supported by a grant of the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences).

References

1. S. J. Mora, E. Odella, G. F. Moore, D. Gust, T. A. Moore, and A. L. Moore, *Acc. Chem. Res.* **2018**, *51*, 445–453.
2. M. T. Huynh, S. J. Mora, M. Villalba, M. E. Tejeda-Ferrari, P. A. Liddell, B. R. Cherry, A-L. Teillout, C. W. Machan, C. P. Kubiak, D. Gust, T. A. Moore, S. Hammes-Schiffer and A. L. Moore, *ACS Cent. Sci.* **2017**, *3*, 372–380.
3. E. Odella, S. J. Mora, B. L. Wadsworth, M. T. Huynh, J. J. Goings, P. A. Liddell, T. L. Groy, M. Gervaldo, L. E. Sereno, D. Gust, T. A. Moore, G. F. Moore, S. Hammes-Schiffer and A. L. Moore, *J. Am. Chem. Soc.* **2018**, *140*, 15450–15460

> IL358. Invited Lecture
Symposium PCHEM-8 Artificial Photosynthesis (Ana Moore)

CHARGE CARRIER DYNAMICS IN CARBON NITRIDE PHOTOCATALYSTS AND HETEROJUNCTIONS

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Presenting Author: Robert Godin
1) The University of British Columbia

Introduction

Carbon nitrides[1] (CN_x) are currently garnering interest as photocatalysts because of their high photocatalytic performance combined with good stability and facile synthesis. However, there still remains significant gaps in our knowledge of the photophysical properties of these organic polymeric materials, severely impeding rational optimization. Determining the pathways and mechanism of photoinduced processes such as charge generation/separation and interfacial redox reaction is key to be able to engineer better CN_x photocatalysts. Notably, the charge carrier population at long timescales (microsecond and longer) are strongly correlated to the observed efficiency, demonstrating the link with charge carrier dynamics.

Results and Discussion

A combined transient absorption spectroscopy (TAS) and time-resolved photoluminescence (tr-PL) study of CN_x provided important insight into their photophysical processes on timescales ranging from femtoseconds to seconds.[2] Notably, over the timescales studied the TAS signal decayed by only 2 orders of magnitudes compared to over 8 orders of magnitude decrease for tr-PL. We developed a simple, quantitative model for the charge carrier dynamics that includes consideration of carrier relaxation into an exponential tail of trap states extending over 1 eV into the bandgap. Relaxation of photogenerated charges into these low energy trap states wastes up to half of the absorbed energy. The resulting loss of driving force for interfacial charge transfer makes proton reduction uncompetitive versus recombination on the microsecond and longer timescales, lowering the photoactivity.

Forming a heterojunction is a common strategy to promote charge separation and charge carrier lifetime to improve the efficiency of photocatalysts. This strategy has also been applied to CN_x, but the organic nature of the semiconductor is rarely taken into account. The distinct molecular interface formed and the impact of processes such as band bending are not well-understood for CN_x heterojunctions. The results of charge carrier dynamics measurements of CN_x/organic[3] and CN_x/inorganic[4] heterojunctions are compared to develop a holistic understanding of CN_x interfaces.

Conclusions

A charge trapping model developed from the observed TAS and tr-PL dacays provides a framework to rationalize the observed photocatalytic efficiencies of CN_x. Forming CN_x heterojunctions with other materials typically doesn't induce a significant change in the charge carrier dynamics over the microsecond – second timescale. Performance differences seem to stem from enhancing rapid charge separation or promoting slow electron transfer to a cocatalyst

There are no conflicts of interest to declare.

References

1. K. Maeda et al. *J. Phys. Chem. C*, 2009, **113**, 4940
2. R. Godin et al. *JACS*, 2017, **139**, 5216
3. H. Kasap, R. Godin et al. *ACS Catal.*, 2018, **8**, 6914
4. A. Crake, K. C. Christoforidis, R. Godin et al., *Appl. Catal. B Environ.*, 2019, **242**, 369

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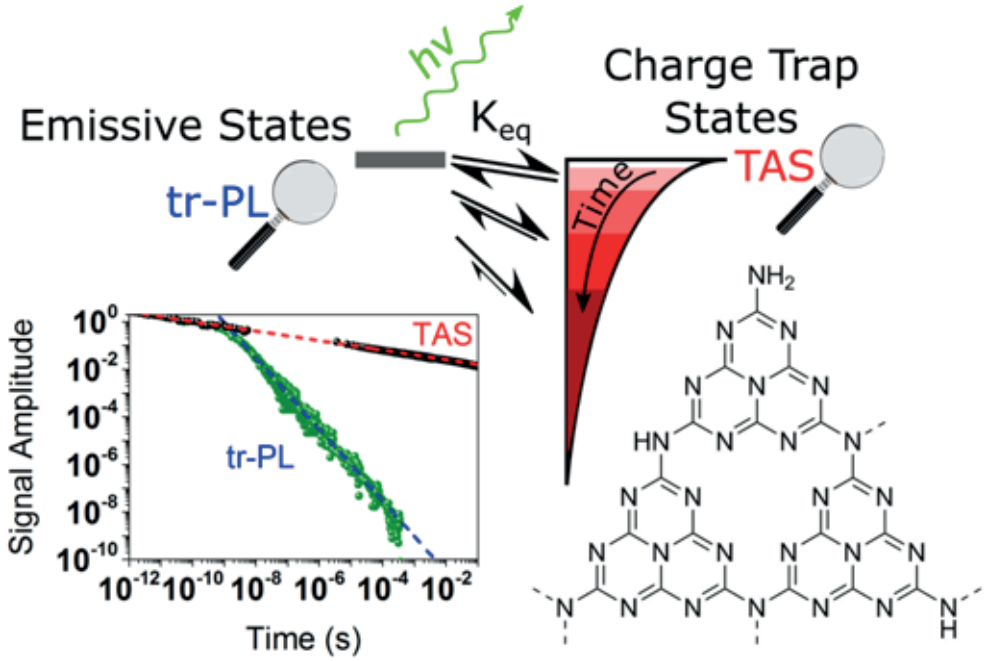
hν
Emissive States
Keq
Charge Trap
States
tr-PL
TAS
Time
Signal Amplitude
TAS
tr-PL
Time (s)
NH2

> IL361. Invited Lecture
Symposium PCHEM-8 Artificial Photosynthesis (Ana Moore)

MOLECULAR MATERIALS FOR ARTIFICIAL PHOTOSYNTHESIS

Authors: Antoni Llobet[1]
Presenting Author: Antoni Llobet
1) Catalna Institute for Chemical Rsearch

The replacement of fossil fuels by a clean and renewable energy source is one of the most urgent and challenging issues our society is facing today, which is why intense research is devoted to this topic recently. Nature has been using sunlight as the primary energy input to oxidize water and generate carbohydrates (a solar fuel) for over a billion years. Inspired, but not constrained by nature, artificial systems[1] can be designed to capture light and oxidize water and reduce protons or other organic compounds to generate useful chemical fuels. In this context this contribution will present how molecular water oxidation catalysts can be anchored on solid supports to generate powerful hybrid electro- and photo-anodes for water splitting.[2]

References

1. (a) R. Matheu, P. Garrido-Barros, M. Gil-Sepulcre, M. Z. Ertem, X. Sala, C. Gimbert-Suriñach, A. Llobet. *Nat. Rev. Chem.* **2019**, DOI: 10.1038/s41570-019-0096-0. (b) R. Matheu, M. Z. Ertem, C. Gimbert-Suriñach, X. Sala, A. Llobet, *Chem. Rev.* **2019**, *119*, 3453–3471.
2. (a) R. Matheu, M. Z. Ertem, J. Benet-Buchholz, E. Coronado, V. S. Batista, X. Sala, A. Llobet, *J. Am. Chem. Soc.* **2015**, *137*, 10786-10795. (b) J. Creus, R. Matheu, I. Peñafiel, D. Moonshiram, P. Blondeau, J. Benet-Buchholz, J. García-Antón, X. Sala, C. Godard, A. Llobet, A. *Angew. Chem. Int. Ed.* **2016,** *55*, 15382-15386. (c) R. Matheu, I. A. Moreno-Hernández, X. Sala, H. B. Gray, B. S. Brunschwig, A. Llobet, A; N. S. Lewis, *J. Am. Chem. Soc.* **2017**, *139,* 11345-11348.

> OC132. Oral Communication
Symposium PCHEM-8 Artificial Photosynthesis (Ana Moore)

ASAPS – ARTIFICIAL SIMPLIFIED AUTOTROPHIC PROTOCELLS

Authors: Emiliano Altamura[1], Paola Albanese[1], Francesco Milano[2], Massimo Trotta[2], Pasquale Stano[3], Fabio Mavelli[1,4]
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The autotrophic property of cells is achieved through a higher order network of molecules, and although it is still unclear how this capability emerged in early cells from an ensemble of non-living molecules. In recent years, scientific attention and experimental efforts have been focused on reconstituting this cell property in artificial systems.[1-4] In this framework, we focus on photosynthetic systems and on the light energy transduction, trying to figure out how the energy flux in cells sustains the autotrophic viability by implementing and modeling an Autotrophic Simplified Artificial Protocell (ASAP). Two main different strategies are followed: the single and the multi compartment approaches (SCA and MCA respectively). In SCA, we try to reconstitute in the lipid membrane of giant unilamellar vesicles (GUVs) all the protein complexes involved in the light phase of bacterial photosynthesis: the reaction center (RC)[5], the coenzyme Q–cytochrome c oxidoreductase (bc1), and the ATP synthase (ATP-syn). GUVs are spherical aqueous compartments closed by a lipid double layer with diameter in the range of tenth of micrometers, that are self-aggregate artificial structures suitable to mimic the cellular morphology. On the other hand, in MCA, instead of extracting each single photosynthetic enzyme from bacteria and reconstituting all of them in the vesicle membrane, we optimize a procedure for extracting cromatophores, small natural organelles (radius 20–50 nm), that contain all the photosynthetic apparatus in their membrane. The cromatophores can be then entrapped in the internal aqueous lumen of GUVs, in order to implement multi-compartment systems able to transduce light energy. Therefore, in both approaches, the final goal is to prepare artificial simplified photo-autotrophic protocells able to convert ADP into ATP molecules driven by light to sustain other fundamental cellular functions such as gene expression and lipid synthesis. In this contribution we describe the steps already done to achieve this ambitious goal following both the mentioned approaches and the further moves to be accomplished in a close future.

References

1. Pohorille A, Deamer D (2002) Artificial cells: Prospects for biotechnology. Trends Biotechnol 20(3):123–128.
2. Noireaux V, Libchaber A (2004) A vesicle bioreactor as a step toward an artificial cell assembly. Proc Natl Acad Sci USA 101(51):17669–17674.
3. Kurihara K, et al. (2011) Self-reproduction of supramolecular giant vesicles combined with the amplification of encapsulated DNA. Nat Chem 3(10):775–781.Luisi P. L., Ferri F., Stano P., (2006), “Approaches to semi-synthetic minimal cells: a review” Naturwissenschaften Vol. 93, 1 – 13.
4. Luisi PL, Ferri F, Stano P (2006) Approaches to semi-synthetic minimal cells: A review. Naturwissenschaften 93(1):1–13.
5. Altamura E., Milano F., Tangorra R.R., Trotta M., Omar H.O, Stano P. and Mavelli F. (2017) Highly oriented photosynthetic reaction centers generate a proton gradient in synthetic protocells. Proc Natl Acad Sci USA, 114(15), 3837-3842.

> OC133. Oral Communication
Symposium PCHEM-8 Artificial Photosynthesis (Ana Moore)

PHOTOSYNTHETIC MULTICOMPARTMENT ARTIFICIAL CELL FOR ATP PRODUCTION

Authors: Emiliano Altamura[1], Paola Albanese[1], Francesco Milano[2], Massimo Trotta[2], Roberto Marotta[3], Pasquale Stano[4], Fabio Mavelli[1, 5]
Presenting Author: Paola Albanese
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Most of complex metabolic reactions in living cells involve different intracellular organelles with different biological functions. The idea to mimic living organisms is on the basis of the construction of the artificial cell.[1-3]The most challenging aspect is the control and the regulation of the biochemical reactions that involve complex membrane protein machinery.

Looking at the photosynthetic bacterium *Rhodobacter sphaeroides*as model organism, here we present a possible way to build a hybrid, artificial and natural, multicompartment system capable to transduce light energy in chemical energy stored in ATP molecules. This system consists of extracted nanometric bacterial vesicles, containing the entire photosynthetic apparatus, i.e. chromatophores[4], used as organelles when entrapped in a micrometric artificial cell-mimicking compartment, a giant lipid unilamellar vesicle (GUV). The photosynthetic apparatus consists of light harvesting complexes LH-I and LH-II, reaction center complexes (RC), coenzyme Q:cytochrome c – oxidoreductase (bc1) and ATP synthase complexes. The peculiarity of this system is its photo-inducibility: continuous infra-red light can trigger cyclic redox reactions producing a proton gradient across the membrane. This proton motive force is afterwards exploited, in presence of ADP and Pi molecules, by the ATP synthase to produce ATP molecules in the external environment.

In this contribution we present an optimized chromatophore extraction procedure that brings to a sample of bacterial vesicles with desired orientation and retained photoactivity quantifying the by chemiluminescence assay, the ATP production under illumination. Afterwards, we encapsulated these photosynthetic organelles in giant lipid vesicles obtaining hybrid photosynthetic artificial cells. After the encapsulation we verified that in absence of ADP molecules, the photosynthetic organelles were able, triggered by infra-red light, to induce an alkalinisation of the giant vesicle water core monitored with a pH-sensitive fluorescent dye, i.e. pyranine. The photosynthetically produced ATP within giant vesicles could be the fuel for sustaining simplified metabolic pathways for the synthesis of macromolecules such as proteins that can confer specific tasks to these artificial protocells.

References

1. Luisi P. L., Ferri F., Stano P., (2006), “Approaches to semi-synthetic minimal cells: a review” *Naturwissenschaften* Vol. 93, 1 – 13.
2. Oberholzer T., Wick R., Luisi P. L., Biebricher C. K. (1995), “Enzymatic RNA replication in self-reproducing vesicles: an approach to a minimal cell” *Biochem. Biophys. Res. Commun.* Vol. 207, 250–257.
3. Walde P. (2010) “Building artificial cells and protocell models: experimental approaches with lipid vesicles” *Bioassays* Vol. 32, 296-303.
4. Altamura E., Mavelli F., Milano F. and Trotta M. (2016), “Photosynthesis Without the Organisms: The Bacterial Chromatophores” *Advances in Bionanomaterials - Lecture Notes in Bioengineering*165-173.

> P168. Poster

Symposium PCHEM-8 Artificial Photosynthesis (Ana Moore)

OPTIMIZING ENZYMATIC PHOTO-REDOX CYCLES BY MEANS OF HYBRID PROTEIN COMPLEXES

Authors: Emiliano Altamura[1], Paola Albanese[1], Francesco Milano[2], Massimo Trotta[2], Fabio Mavelli[1,3]
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The aim of this contribution is to describe the implementation and the optimization of a hybrid enzymatic system that performs light energy transduction through a photo redox cycle by mimicking the first step of the bacterial photosynthesis. In nature, this step takes place in the cytoplasmic membrane of photosynthetic bacteria[1]. The energy transduction starts with the bacterial photosynthetic reaction center (RC_B) and the ubiquinol oxidase ($bc1_B$) that catalyzes a redox photo-cycle to convert the light energy in a transmembrane pH gradient across the cytoplasmic membrane. In the redox photo-cycle, RC_B absorbs two photons and catalyzes the oxidation of cytochrome cyt^{2+} and the reduction of quinone to quinole:

$$Q + 2H^+ + 2cyt^{2+} \rightarrow QH_2 + 2cyt^{3+}$$

by taking two protons from the internal milieu. Conversely, $bc1_B$ catalyzes the backward reaction with the net translocation of $4H^+$ to the external environment of the cytoplasmic membrane. The pH gradient is eventually exploited by ATP-synthase for the conversion of ADP in ATP using an endogenous phosphate.

On the other hand, in mammalian cells, the cytochrome complex ($bc1_M$) is an energy-transducing, electron-transfer enzyme located in the inner mitochondrial membrane of oxygen-utilizing eukaryotic cell, where it takes part in cell respiration. Similarly to $bc1_B$, $bc1_M$ converts the energy associated with electron transfer from ubiquinol to cytochrome c into an electrochemical proton gradient across the membrane in which the enzyme resides: *Proton motive Q-cycle*.

The photo-redox cycle has been studied in an artificial micellar suspension[2] comparing the performance of the bacterial $bc1_B$ complex with the version extracted from bovine heart cells $bc1_M$ showing that the light transduction can be enhanced by coupling a hybrid enzymatic system and by tuning the enzymatic ratio $RC_B/bc1_M$. This work represents a step forward towards the implementation of a highly efficient photo-autotrophic artificial cell[3].

References

1. Altamura E., Mavelli F., Milano F. and Trotta M. (2016), “Photosynthesis Without the Organisms: The Bacterial Chromatophores” *Advances in Bionanomaterials - Lecture Notes in Bioengineering* 165-173.
2. Altamura E, Fiorentino R, Milano F, Trotta M, Palazzo G, Stano P, Mavelli F. (2017), “First moves towards photoautotrophic synthetic cells: In vitro study of photosynthetic reaction centre and cytochrome bc1 complex interactions”. *Biophysical Chemistry*, 229, 46-56.
3. Altamura E., Albanese P., Marotta R., Stano P., Milano F., Trotta M., Mavelli F. (2019) “Towards the Synthesis of Photo-Autotrophic Protocells” *Computational Intelligence Methods for Bioinformatics and Biostatistics*, 186-199.

> IL363. Invited Lecture

Symposium PCHEM-9 Molecular Modelling and Simulation in photobiological systems (Antonio Monari)

PHOTOSENSITIZATION AND PHOTOLESIONS PRODUCTION AND EVOLUTION IN DNA

Authors: Antonio Monari[1]
Presenting Author: Antonio Monari
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The interaction between light and different biological systems represents a crucial phenomenon in biology and is notably responsible of many fundamental outcomes related to signaling as well as energy conversion and storage.

Light is indeed essential to assure life as we know it. On the other hand light may also represent an external stress source producing harmful effects related most notably to cancer development.

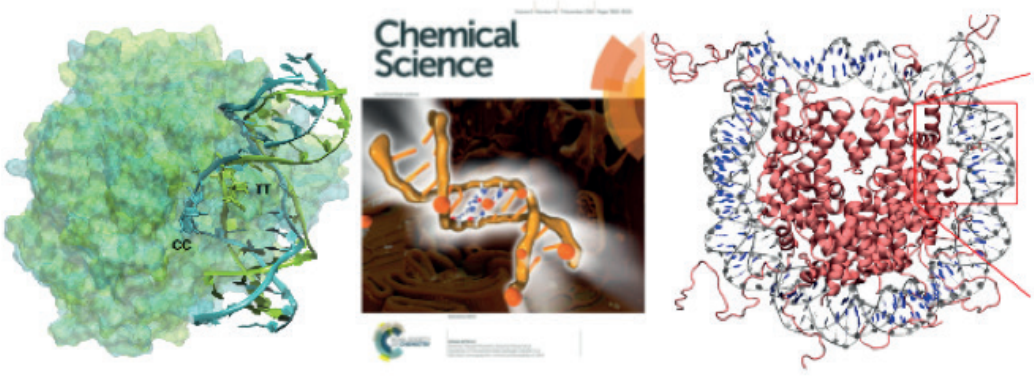
In this talk we will, through a series of examples mostly related to DNA photolesions induction and repair, illustrate the crucial role played by molecular modeling and simulation in elucidating such processes. We will also illustrate the crucial need of tackling multiscale phenomena taking into account at the same time the complex electronic structures and their interplay with dynamical sampling and time evolution in complicated biological macrostructures.

By using appropriate and high level molecular modeling and simulation strategies we will prove that the scientific field is nowadays ready to provide answers to, and hence rationalize, biological questions related most notably to mutation, DNA replication, and cell resistance to stress.

Hence, we will demonstrate that molecular simulations is leading us into the age of *in silico molecular* photobiology.

References

1. Francés-Monerris et al. (2018) *Chem. Sci. 9,* 7885.
2. Dehez et al. (2017) *Nucleic Acids Res. 45* 3654-3662
3. Bignon et al.(2017) *Nature Sci. Rep. 7,* 8885
4. Gattus et al. *Phys. Chem. Chem. Phys. 19*, 23187
5. Gattuso et al. (2016) *Phys. Chem. Chem. Phys. 18,* 18598
6. Bignon et al. (2016) *Nucleic Acids Res. 44,* 8588
7. Gattuso et al. (2016) *J. Phys. Chem. Lett. 7,* 3760
8. Fumanal et al. (2018) *Chem – Eur. J. 24*, 14425
9. Francés-Monerris et al. (2018) *Phys. Chem. Chem. Phys. 20,* 25666



> IL364. Invited Lecture

Symposium PCHEM-9 Molecular Modelling and Simulation in photobiological systems (Antonio Monari)

QM/MM INSIGHTS INTO THE BINDING AND CHEMISTRY OF DNA PHOTOSENSITIZERS

Authors: Elise Dumont[1], Antonio Monari[2]
Presenting Author: Elise Dumont
1) Univ Lyon, Ens de Lyon, CNRS UMR 5182, Université Claude Bernard Lyon 1, Laboratoire de Chimie, F69342, Lyon, France 2) Laboratoire de Physique et Chimie Théoriques , Université de Lorraine

DNA photosensitization and covers a large range of damage, with major biological consequences [1]. Photosensitizers span a large of compounds, whereas intensive research efforts are ongoing to develop efficient singlet oxygen targetting G-quadruplexes [2]. I will show how molecular dynamics simulations can assess the binding of small drugs onto DNA and provide an accurate picture of the electronic phenomena undergoing triplet-triplet energy transfer or singlet oxygen generation, taking the example of benzophenone [3,4] and palmatine [5,6] respectively.

Acknowlegments: ED thanks the funding agency LABEX PRIMES (ANR-11-LABX-0063) of Université de Lyon (within the program “Investissements d’Avenir” ANR-11-IDEX-0007)

References

[1] B. Epe, Photochem. Photobiol. Sci. 2012, 11:98
[2] V. Grande, C.-A. Shen, M. Deiana, M. Dudek, J. Olesiak-Banska, K. Matczyszyn, F. Wurthner, Chem. Sc., 2018, 9:8375
[3] M. C. Cuquerella, V. Lhiaubet-Vallet, J. Cadet, M. A. Miranda, Acc. Chem. Res., 2012, 45:1558
[4] E. Dumont, M. Wibowo, D. Roca Sanjuan, M. Garavelli, X. Assfeld, A. Monari, J. Phys. Chem. Lett., 2015 6:76
[5] E. Dumont, A. Monari J Phys Chem B 2014, 119:410
[6] E. Dumont, A. Monari, Front. Chem., 2015, 3:43

> IL365. Invited Lecture

Symposium PCHEM-9 Molecular Modelling and Simulation in photobiological systems (Antonio Monari)

PHOTOPHYSICS AND PHOTOCHEMISTRY OF GUANINE QUADRUPLEXES: INSIGHTS FROM QUANTUM MECHANICAL CALCULATIONS

Authors: Lara Martinez Fernández[2], Haritha Asha[1], Akos Banyasz[3], Dimitra Markovitsi[4], Roberto Improta[1]
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Guanine-rich DNA sequences can adopt a peculiar fold, a Quadruple helix, where four Guanine (G) bases are arranged in planar structures, the tetrads, stabilized by Hoogsteen-type hydrogen bonds.[1] G-Quads are involved in key cellular processes and have emerged as promising therapeutic targets. G has a low oxidation potential, making G-Quad particularly vulnerable to oxidative damage, not only caused by the action of other molecules, but also direct, i.e. caused by the mere absorption of UV light.[2,3] Following 266 nm irradiation, different G-quads, i.e. a human telomeric tract and TG_4T, undergo to one-photon ionization with noticeable quantum yield (~10^{-3}).[4,5] Fluorescence is also enhanced by quadruplex formation.[6] We here show that Quantum mechanical (QM) calculations can provide an atomistic description of the processes triggered by the absorption of UV light, reproducing and assigning the experimental optical and electronic circular dichroism spectra.

Our QM computational approach is based on DFT and TD-DFT calculations, by using long-range corrected functionals. Solvent effects are included by means of a mixed implicit (based on the Polarizable Continuum Mode)/explicit approach. In order to describe G-Quads we resorted to mixed Quantum Mechanics/Molecular Mechanics (QM/MM) calculations, where the backbone is treated at the MM level, whereas the bases and the inner cations at the QM level.

When applied to the study of the main Guanine centered radicals our approach provide absorption spectra in fair agreement with the experiments both in solution [7] and within G-quads [4,5], thus helping the interpretation of the time resolved spectra connected to one photon ionization. We study different Quadruplex topologies,[4,5,8] that of the human telomere [4,5,9], of parallel G-quads [5,8,9] and that of thrombin binding aptamer [8]. Absorbing excited states are delocalized over multiple bases, whereas emission involves a stacked guanine dimer or a monomer. In this way, we can provide a full assignment of the absorption and emission spectra of G-quads. The deactivation pathways are strongly modulated by the Quadruplex topology and, strikingly, for the human telomere we also identify a reactive funnel leading to dimerization of two stacked guanines.[9]

Acknowledgements

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References

1] R. Hänsel-Hertsch et al *Nature Rev. Mol. Cell Biol.* **2017,** *18*, 279.
2] L.P. Candeias & S. Steenken, *J.Am. Chem. Soc.* **1992,** *114*, 699.
3] M. Gomez-Mendoza, et al. *J. Phys. Chem. Lett.* **2016,** *7*, 3945.
4].A. Banyasz,et al. *J.Am. Chem. Soc* **2017,** *139*, 10561.
5] L. Martínez-Fernández et al. *Chem. Eur. J.* **2018,** *24*, 15185.
6] P. Changenet-Barret, et al. Electronic Excitations in Guanine Quadruplexes in Photoinduced Phenomena in Nucleic Acids II: Springer International Publishing: Cham, **2015**; 183.
7] L. Martìnez Fernàndez, et al *ChemPhotoChem* accepted for the publication
8] R. Improta *Chem. Eur. J.* 2014, 20, 8106.
9] L. Martìnez Fernàndez et al. to be submitted.

> IL366. Invited Lecture

Symposium PCHEM-9 Molecular Modelling and Simulation in photobiological systems (Antonio Monari)

THE MOLECULAR MECHANISMS OF LIGHT ADAPTION IN LIGHT-HARVESTING COMPLEXES OF PURPLE BACTERIA REVEALED BY A MULTISCALE MODELING

Authors: Michele Nottoli[1], Felipe Cardoso Ramos[1], Lorenzo Cupellini[1], Benedetta Mennucci[1]
Presenting Author: Michele Nottoli
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Photosynthetic purple bacteria tune the light harvesting in response to the light intensity using different mechanisms. One of the strategies is to synthesize different major light harvesting complexes (LH2) presenting different spectroscopic properties and energy transfer rates to the reaction center. In this talk, we present a computational study of the microscopic origin of the observed spectroscopic differences. The study is based on three different LH2 complexes grown in different light conditions. We used a combination of classical molecular dynamics, multiscale quantum calculations and an excitonic approach to predict the absorption spectra of the three systems. Then we analyzed the mechanisms governing the light adaptation finding that the different hydrogen bond network present in the three systems plays a central role, through a tuning of the excitation energies of the individual bacteriochlorophylls and their relative geometrical fluctuations which finally lead to different couplings.

> IL367. Invited Lecture

Symposium PCHEM-9 Molecular Modelling and Simulation in photobiological systems (Antonio Monari)

MODELING RHODOPSIN PHOTOSENSITIZATION TO EXPLAIN RED VISION

Authors: Marco Marazzi[1,2], Hugo Gattuso[3], Daniel Roca-Sanjuán[4], Francois Dehez[5], Antonio Monari[5]
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Rhodopsins are involved in the primary event of mammalian vision, thanks to a direct light-initiated cis-trans isomerization of the retinal chromophore. In the deep-sea, where light intensity is much lower than Earth's surface, the use of photosensitizers has been investigated as a possible source of indirect visual signal transduction pathway: molecules derived from chlorophyll – hence porphyrin-like – can absorb longer-wavelength light and transfer the gained energy to shorter wavelength visual pigments, as retinal. Porphyrins were also administrated as drugs for photodynamic therapy in these last years, reporting as side effect enhanced visual sensitivity, *i.e.* night vision. This drawback was turned in recent years into a benefit, since ophthalmic preparations were proposed to treat night blindness [1].

Here, we present the multi-scaling computational study of chlorin e6, a porphyrin-like photoactive drug, in contact with bovine rhodopsin including a model lipid bilayer. The interaction modes between chlorin e6 and rhodopsin will be elucidated and discussed on the basis of previously reported experimental studies [2,3].

Further, the modeled optical properties (including one- and two-photon absorption, fluorescence and phosphorescence [4]) will be related to the photosensitization pathways to activate retinal and finally explain red and near infra-red vision.

Especially, long-range Förster-type and short-range Dexter-type energy transfer mechanisms will be considered [5], as well as the possible active role of singlet oxygen, commonly produced by irradiated porphyrin-like compounds [6]

References

[1] Álvarez R., Vaz B., Gronemeyer H., de Lera Á. R., *Chem. Rev.* **2014**, *114,* 1-125.
[2] Washington I., Brooks C., Turro N. J., Nakanishi K., *J. Am. Chem. Soc.* **2004**, *126*, 9892–9893.
[3] Isayama T., Alexeev D., Makino C. L., Washington I., Nakanishi K., Turro N. J., *Nature* **2006**, *443*, 649–649.
[4] Gattuso H., Monari A., Marazzi M., *RSC Advances* **2017**, *7*, 10992-10999.
[5] Valentini A., Nucci M., Frutos L. M., Marazzi M. *ChemPhotoChem* **2019**, submitted.
[6] Marazzi M., Gattuso H., Dehez F., Monari A., *J. Am. Chem. Soc.* **2019**, in preparation.

> IL368. Invited Lecture

Symposium PCHEM-9 Molecular Modelling and Simulation in photobiological systems (Antonio Monari)

PHOTO- AND CHEMI-INDUCED EXCITED-STATE CHEMISTRY OF INTEREST IN BIOLOGY AND MEDICINE: DNA REPAIR, LUMINOL, AND NIGHT VISION

Authors: Daniel Roca-Sanjuán[1], Javier Carmona-García[1], Miriam Navarrete-Miguel[1], Antonio Francés-Monerris[2], Angelo Giussani[1]
Presenting Author: Daniel Roca-Sanjuán
1) Universitat de València 2) Université de Lorraine

Ligh-matter interaction gives rise to many physical and chemical phenomena of relevance for Life and Medicine, for example, the UV-induced production of DNA lesions and DNA photo-repair mechanisms, the process of vision, or the mechanisms of photodynamic therapy. All these phenomena involve more than one electronic state and in many occasions state crossings, such as conical intersections and singlet-triplet crossings. The computational study of this excited-state chemistry requires high-level methodological approaches able to deal with the multiple electronic configurations characterizing the state crossings and fast enough to explore the distinct plaussible chemical mechanisms.

By efficiently combining density functional theory and multiconfigurational quantum chemistry, we have studied during the last years the ring opening of azetidines induced by photo-oxidizers/reducers, the chemiluminescence mechanism of luminol, and the activation of retinal (isomerization) in the darkness. The most important findings shall be discussed in this contribution describing the electronic structure and chemical nature of the phenomena and mentioning the implications for biology and medicine.

> IL369. Invited Lecture

Symposium PCHEM-9 Molecular Modelling and Simulation in photobiological systems (Antonio Monari)

THEORETICAL STUDIES OF BIOLUMINESCENCE OF FIREFLIES: HOW TO PREDICT THE COLOR?

Authors: Isabelle Navizet[1], Cristina Garcia-Iriepa[1], Romain Berraud-Pache[1], Madjid Zemmouche[1]
Presenting Author: Isabelle Navizet
1) MultiScale Modelling and Simulation laboratory (MSME) at Paris-Est Marne la Vallee University

The emitting light in fireflies arises from the electronic relaxation of oxyluciferin, an organic compound resulting from the oxidation of the D-luciferin substrate inside an enzyme called luciferase.

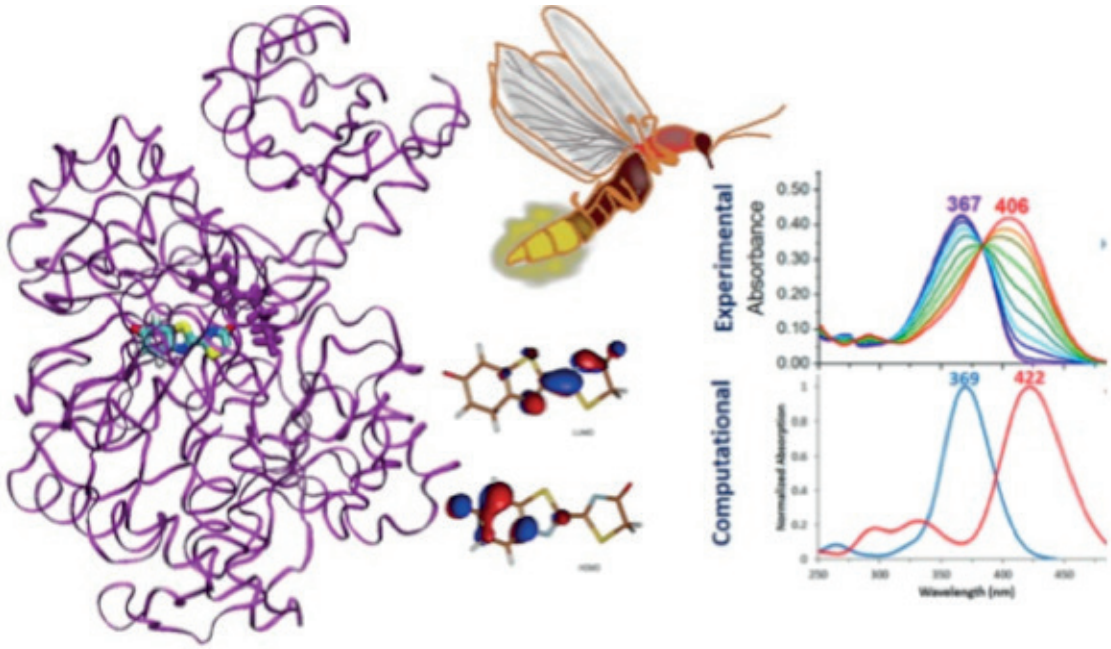
As the fireflies' bioluminescent system is already used as a marker in biology, man needs to understand what are the chemical and physical important factors responsible for the emitted light's color. In order to have insight of the mechanism of the light emission, both experimental and theoretical joint studies have been performed.

I will present here how theoretical tools can give insight to the color modulation in the fireflies' bioluminescence. In order to theoretically study such systems, the use of quantum mechanical/molecular mechanical (QM/MM) methods is required. Accurate QM level is needed for dealing with electronic transition and charge transfer phenomena. Taking into account the surrounding protein at the MM level is essential in order to understand the color modulation and influence of the enzyme.

The presentation will present briefly the methods used and will discuss examples of how theoretical studies can give complementary insights to the experimental results for the understanding of such complex phenomena. Fluorescence and bioluminescence phenomena will be compared. Influence of the surrounding environment (notably mutation in the luciferase[1]) or artificial modification of the wild type emitter[2],[3] will be presented.

References

1. Navizet,I; Liu Y-J; Ferré N; Xiao H-Y; Fang W-H; Lindh R, *J. Am. Chem. Soc.* **2010** (132), 704-712.
2. Berraud-Pache R and Navizet I, *PCCP*, **2016** (18), 27460 – 27467.
3. García-Iriepa C; Gosset P; Berraud-Pache R; Zemmouche M; Taupier G; Dorkenoo K D; Didier P; Léonard J; Ferré N and Navizet I, Simulation and analysis of the spectroscopic properties of oxyluciferin and its analogues in water, *JCTC* **2018**, 14, 2117-2126.



> OC134. Oral Communication

Symposium PCHEM-9 Molecular Modelling and Simulation in photobiological systems (Antonio Monari)

A QSPR STUDY OF TRIPLET STATE PHOTOGENERATION AND SINGLET OXYGEN (1ΔG) FORMATION BY ORGANIC PHOTOSENSITIZERS

Authors: Andrey Buglak[1], Alexei Kononov[1]
Presenting Author: Andrey Buglak
1) Saint Petersburg State University

Quantitative structure-property relationship (QSPR) is a procedure of building models that allows to predict various properties of compounds based on their chemical structure. QSPR is used in photochemistry to predict the maximum absorption wavelength, fluorescence intensity, photoinduced toxicity, and quantum yield of photolysis.

The goal of our study was to develop the methods for the pre-synthetic prediction of the photodynamic activity of photosensitizers. There exist several works in which QSPR models “structure - photodynamic activity” were created based on the analysis of experimental data obtained in vitro or in vivo using such methods as, for example, 3T3 NRU test. However, models built on the basis of in vivo and in vitro experimental data have some limitations due to the usage of (a) a concrete biological object and (b) a certain finite group of compounds. The approach suggested in our study is more universal since we use quantum yield of singlet oxygen formation (Φ_Δ) and quantum yield of triplet state generation (Φ_T), which are fundamental photophysical properties known for a wide range of chemicals, as a dependent variable in QSPR.

Φ_T depends on intersystem crossing (ISC) rate which is governed by spin-orbit coupling (SOC). In quantum chemistry approximate SOC operators and state-of-the-art theoretical methods are used for evaluation of ISC rates. Since it is required to perform virtual screening of large libraries of compounds for pharmaceutical purposes, calculation of SOC operators seems inappropriate in this case. The alternative strategy is the application of QSPR methodology. To our knowledge until our work there have been no attempts to predict the values of Φ_Δ and Φ_T using the QSPR methodology.

Psoralens and pterins are two groups of organic heterocyclic compounds widely used in biochemistry and photochemistry. Φ_T was significantly correlated with triplet state energy (R^2 = 0.627) of psoralens. The best QSPR model for psoralens possessed high internal stability (q^2=0.865) and high predictive ability towards the test set (pred_R^2 = 0.897) [1]. Φ_Δ of pterins was influenced by their ionization potential, electronegativity, as well as some minor parameters [2]. We built a local QSPR model which allowed us to predict Φ_Δ with high q^2 and pred_R^2 values: q^2 =0.881 and pred_R^2=0.873.

In silico models for the virtual prediction of the photosensitizing activity can be used to create new photodynamic agents for antimicrobial and anticancer photodynamic therapy. The next step in our study is to apply the QSPR methodology to the analysis of $^1O_2$ production by metalloporphyrins and metal nanoclusters.

References

1. Buglak AA, Kononov AI. Triplet state generation by furocoumarins revisited: a combined QSPR/DFT approach. New J Chem. 2018;42:14424-14432.
2. Buglak AA, Telegina TA, Kritsky MS. A quantitative structure-property relationship (QSPR) study of singlet oxygen generation by pteridines. Photochem Photobiol Sci. 2016;15(6):801-811.

> P169. Poster

Symposium PCHEM-9 Molecular Modelling and Simulation in photobiological systems (Antonio Monari)

DESIGNING A K+ CONDUCTING KR2 CHANNEL VARIANT

Authors: Enrico Peter[1]
Presenting Author: Enrico Peter
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Krokinobacter eikastus rhodopsin 2 (KR2) was the first discovered light-driven outward directed Na^+ pump and is especially of interest for the development of light-activated cation channels. Here, we demonstrate an enzyme evolution approach supported by theory to design a K^+ selective KR2 channel. Recent electrophysiological experiments suggest single mutations establishing K^+ selectivity and a channel-like characteristic, but the resulting K^+ selectivity remains unsatisfactory. Moreover, the K^+ conductance is highly pH dependent and optimal at alkaline extracellular pH. Based on these findings, a KR2 mutant library was created to identify several K^+ conducting variants with the help of a selection system using a K^+ uptake deficient *E.coli* strain. Additionally, quantum chemistry, molecular dynamics simulations and electrostatics calculations using Karlsberg2+ support the experiments in finding putative mutation sites to further optimize the selectivity and pH dependency.

> P170. Poster

Symposium PCHEM-9 Molecular Modelling and Simulation in photobiological systems (Antonio Monari)

COMPUTATIONAL STUDY OF LH2 COMPLEX LIGHT AND DARK ADAPTATION IN PURPLE BACTERIA

Authors: Felipe Cardoso Ramos[1], Michele Nottoli[1], Lorenzo Cupellini[1], Benedetta Mennucci[1]
Presenting Author: Felipe Cardoso Ramos
1) Department of Chemistry, University of Pisa

The light-harvesting (LH) apparatus of a typical photosynthetic purple bacteria is composed of the LH1 and LH2 complexes, which act together in the absorption and transfer of excitation energy to the reaction center (RC). The LH2 complexes are circular membrane proteins formed of nine dimeric apoproteins (*α* and *β* chains), each bound to one carotenoid (Car) and three bacteriochlorophyll a (Bchl) molecules for a total of 27 BChls arranged in two rings made of 9 and 18 BChls. Purple bacteria express LH2 complexes with different αβ apoproteins depending on the light intensity, which allows them to adapt to the luminosity conditions [1]. In particular, the species *Rhodopseudomonas acidophila* (*Rps. acidophila*) expresses LH2 complexes with absorption peaks at 800 and 850 nm (B800-850 complex) when in high light (HL) conditions, but when in low light (LL) conditions they are replaced by complexes that absorb at 800 and 820 nm (B800-820 complex) [2]. The computational simulation of LH complexes absorption spectra is crucial to a full comprehension of their structure-function relationship [2,3]. Here, we performed classical molecular dynamics (MD) of different LH2 complexes from purple bacteria in lipid membranes to generate equilibrated systems. Then we extracted structures from MD trajectories and used it in hybrid quantum mechanics/molecular mechanics calculations using a polarizable embedding MM formulation (QM/MMpol). Once we had calculated the required quantities (excitation energies for each BChl and electronic coupling for each pair of BChls), we reconstructed the Hamiltonian of the multichromophoric complexes using an excitonic approach and finally simulated their absorption spectra. The results correctly found that the LL complexes have blue-shifted B850 Bchls, in agreement with spectroscopic data. Moreover, they allow us an in-depth explanation about the mechanism that govern the structural adaptation of purple bacteria to LL conditions.

References

[1] Cogdell, R. J., Gall, A., & Köhler, J. (2006). The architecture and function of the light-harvesting apparatus of purple bacteria: From single molecules to in vivo membranes. *Quarterly Reviews of Biophysics*, 39(3), 227–324. DOI: 10.1017/S0033583506004434.
[2] Curutchet, C., & Mennucci, B. (2016). Quantum Chemical Studies of Light Harvesting. *Chemical Reviews*, 117(2), 294–343. DOI: 10.1021/acs.chemrev.5b00700.
[3] Nottoli, M., Jurinovich, S., Cupellini, L., Gardiner, A. T., Cogdell, R., & Mennucci, B. (2018). The role of charge-transfer states in the spectral tuning of antenna complexes of purple bacteria. *Photosynthesis Research*, 1–12. DOI: 10.1007/s11120-018-0492-1.

> P171. Poster

Symposium PCHEM-9 Molecular Modelling and Simulation in photobiological systems (Antonio Monari)

STRUCTURAL FACTORS DETERMINING THE ABSORPTION SPECTRUM OF THE CHANNELRHODOPSIN CHIMAERA C1C2

Authors: Suliman Adam[1], Christian Wiebeler[1], Ana-Nicoleta Bondar[2], Igor Schapiro[1]
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Channelrhodopsins (ChR) are light-activated ion channels with a retinal chromophore covalently attached to a lysine amino acid residue via a protonated Schiff base.[1] After absorbing a photon the retinal isomerises, which starts a photocycle that leads to cations entering the cell, thereby causing a depolarization of the plasma membrane.[2] ChRs have found application in optogenetics, where cells or whole organisms are controlled by light-sensitive ion channels.[2-3] We have investigated factors that determine the absorption maximum of the retinal chromophore inside the ChR chimaera C1C2.[4] Our aim is to derive an understanding at the molecular level in order to be able to tailor the absorption wavelength by mutations. We have sampled the geometries of membrane-embedded C1C2 and computed absorption spectra for 3000 snapshots. Our calculated absorption maximum of 524 nm is within 0.3 eV of the experimental value of 470 nm.[4] Dissection of our spectra according to different structural and electronic determinants reveals that protonation of the counterion E162 causes a red shift of ~20 nm. Moreover, the absorption maximum is strongly correlated with the bond order alternation of the retinal (r = 0.8). Lastly, we conclude that differences in the hydrogen-bonding networks involving the retinal Schiff base have a negligible effect on the absorption spectrum.

References

1. Nagel, G.; Ollig, D.; Fuhrmann, M.; Kateriya, S.; Musti, A. M.; Bamberg, E.; Hegemann, P., Channelrhodopsin-1: a light-gated proton channel in green algae. Science 2002, 296 (5577), 2395-8.
2. Berthold, P.; Tsunoda, S. P.; Ernst, O. P.; Mages, W.; Gradmann, D.; Hegemann, P., Channelrhodopsin-1 initiates phototaxis and photophobic responses in chlamydomonas by immediate light-induced depolarization. The Plant cell 2008, 20 (6), 1665-77.
3. Yizhar, O.; Fenno, L. E.; Davidson, T. J.; Mogri, M.; Deisseroth, K., Optogenetics in neural systems. Neuron 2011, 71 (1), 9-34.
4. Kato, H. E.; Zhang, F.; Yizhar, O.; Ramakrishnan, C.; Nishizawa, T.; Hirata, K.; Ito, J.; Aita, Y.; Tsukazaki, T.; Hayashi, S.; Hegemann, P.; Maturana, A. D.; Ishitani, R.; Deisseroth, K.; Nureki, O., Crystal structure of the channelrhodopsin light-gated cation channel. Nature 2012, 482 (7385), 369-74.

> P172. Poster

Symposium PCHEM-9 Molecular Modelling and Simulation in photobiological systems (Antonio Monari)

COMPUTATIONAL STUDIES ON SPECTROSCOPIC PROPERTIES OF CAROTENOIDS IN PROTEIN ENVIRONMENT

Authors: Mattia Bondanza[1], Lorenzo Cupellini[2], Daniele Loco[3], Benedetta Mennucci[1]
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Many different carotenoids with a wide variety of functions are found in nature. In recent years, a lot of effort was devoted to the study of carotenoid-protein complexes[1]; these complexes play a central role in photosynthesis[2], photo-protection[3] and other processes that involve interaction between soft-matter and light.

Often the spectroscopic properties of carotenoids are very sensitive to the environment. Understanding the relationship between the biological matrix and the optical activity of the complex is a highly challenging topic, which can provide interesting insight into the biological function of these structures.

In our work we exploit novel theoretical and computational methodologies in order to propose a rationale behind the experimental data collected from carotenoid-protein complexes. A wild range of techniques, from conventional and enhanced classical molecular dynamics to multiscale quantum-mechanical calculations, is applied in order to overcome the computational difficulties imposed by these large systems that are characterized by both fast and slow motion coupled with the excitation.

In the poster I will present some results of the application of these methods to the case of Orange Carotenoid Protein (OCP) , Red Carotenoid Protein (RCP) and Helical Carotenoid Protein (HCP2).

References

[1]Kerfeld, C. A. et al. New Phytologist **2017**, 215, 937–951.
[2]Carbonera, D. et al. Current Protein & Peptide Science **2014**, 15, 332–350.
[3]Leverenz, R. L. et al. Science **2015**, 348, 1463–1466.

> P173. Poster

Symposium PCHEM-9 Molecular Modelling and Simulation in photobiological systems (Antonio Monari)

A DIODE-LASER BASED NANOSECOND LASER FLASH PHOTOLYSIS SYSTEM FOR THE DETECTION OF REACTIVE INTERMEDIATES

Authors: Adrián Pinilla-Sánchez[1], Roger Bresoli-Obach[1,2], Santi Nonell[1]
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Laser flash photolysis technique is a spectroscopic tool which is useful to study light-induced reactive intermediate species. The technique is based in the excitation of the sample using a nanosecond-pulsed laser. Then, excited states or metastable species are generated, which are detected by changes in the sample absorbance. Exactly, according to lambert beer law these ΔAbs can be related with the concentration of the generated species[1,2]. Here we present a modification of nanosecond laser flash photolysis system. Specifically, it uses CW laser as monitoring beam, replacing the commonly used white xenon arc lamp[3,4]. The higher stability, coherence and monochromaticity of the CW lasers in comparison with the xenon lamp, allows to eliminate the monochromator and to substitute the photomultiplier for a cheap silicon PIN photodiode as a detector.

The changes introduced into the system boosts the signal to noise ratio in comparison with the standard system, reducing the detection limit and the power of the pump beam. This reduction in power allows the use of an OPO as a pump beam so to tune the excitation wavelength in the 400-700 nm range. The applicability and reliability of the device is demonstrated for various probe wavelengths, from the visible to near-infrared, by the investigation of excited-state decay and photoinduced bimolecular reactions, putting a specially emphasis on light-sensitive molecules and biomolecules, such as fluorescent proteins, like miniSOG. Furthermore, the changes introduced in the laser flash photolysis system reduces the economical cost considerably, making this technique more accessible for the scientific community.

Acknowledgements
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References

(1) Porter, G. N. Flash Photolysis and Spectroscopy. A New Method for the Study of Free Radical Reactions. *Proc. R. Soc. London. Ser. A. Math. Phys. Sci.* **1950**, *200* (1061), 284–300.
(2) Lindqvist, L. Utilisation d'un Laser B Emission Ultraviolette Pulse in Photolysedclairs: Etude de l'etat Triplet de l'acridme. *C. R. Hebd. Seances Acad. Sci.* **1966**, *263* (Serie C), 852–854.
(3) Zewail, A. H. Femtochemistry : Atomic-Scale Dynamics of the Chemical Bond †. *J. Phys. Chem. A* **2000**, *104* (24), 5660–5694.
(4) Schmidhammer, U.; Roth, S.; Riedle, E.; Tishkov, A. A.; Mayr, H. Compact Laser Flash Photolysis Techniques Compatible with Ultrafast Pump-Probe Setups. *Rev. Sci. Instrum.* **2005**, *76* (9), 093111.

> IL371. Invited Lecture
Symposium PCHEM-10 Femtobiology (Dongping Zong)

THE MECHANISM OF PHOTOSYNTHETIC WATER-SPLITTING REVEALED BY FEMTOSECOND X-RAY FREE ELECTRON LASERS

Authors: Jian-Ren Shen[1], Fusamichi Akita[1, 2], Yoshiki Nakajima[1], Michihiro Suga[1, 2]
Presenting Author: Jian-Ren Shen
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Femtosecond X-ray free electron lasers (XFELs) have opened a new avenue for structural studies of proteins and enzymes in their working states. We have employed XFELs provided by SPring-8 angstrom compact free-electron laser (SACLA) to solve the radiation damage-free structure[1] as well as intermediate state structures[2] of photosystem II (PSII), a huge membrane protein complex with a total molecular weight of 700 kDa for a dimer. PSII catalyzes light-induced water-splitting at its catalytic center, which was determined to be a Mn_4CaO_5-cluster organized in a distorted cubane form[3], through four sequential steps via an S*i*-state cycle (S_i, i = 0-4). Before the reaction starts, the catalyst resides in the S_1-state, which is dark-stable. Our damage-free structure by XFELs has revealed the detailed arrangement of each atoms and inter-atomic distances within the Mn_4CaO_5-cluster in the S_1-state[1]. We further employed the XFELs at SACLA to solve the structures of the intermediate S-states by a pump-probe approach using the serial femtosecond X-ray crystallography (SFX) method. As a result, we obtained the structure of PSII in its 2-flashes induced S_3-state[2], and found that a new oxygen designated O6, was inserted in a position close to O5. Since O5 is a unique oxo-bridged oxygen that is weakly bound to the nearby Mn ions in the S_1-state, our results suggested a possible mechanism for the formation of O=O bond between O5 and O6. In order to remove possible uncertainties in the O5-O6 distance we reported and uncover the molecular mechanism of O=O bond formation, we used XFELs to solve the structures of the intermediate S_3 as well as S_2-states at improved resolutions. The results we obtained allow us to determine the detailed molecular mechanism for O=O bond formation in the Mn_4CaO_5-cluster of PSII.

Acknowledgments
We thank a number of collaborators who contributed to in this work but not listed here due to the limited space.

References

1. Suga, M. *et al.* Native structure of photosystem II at 1.95 Å resolution viewed by femtosecond X-ray pulses. *Nature* **517**, 99-103, (2015).
2. Suga, M. *et al*. Light-induced structural changes and the site of O=O bond formation in PSII caught by XFEL. *Nature* **543**, 131-135, (2017).
3. Umena Y., Kawakami K., Shen J.-R., Kamiya N. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* **473**, 55-60, (2011).

> IL372. Invited Lecture
Symposium PCHEM-10 Femtobiology (Dongping Zong)

PRIMARY ISOMERIZATION REACTIONS IN PHOTOACTIVE YELLOW PROTEIN IN CRYSTAL AND SOLUTION PHASES SHOW DISTINCT RATES AND STRUCTURAL INTERMEDIATES

Authors: John Kennis[1], Enis Arik[1], Patrick Konold[1], Joern Weissenborn[1], Jos Arents[2], Klaas Hellingwerf[2], Ivo van Stokkum[1], Marie-Louise Groot[1]
Presenting Author: John Kennis
1) Vrije Universiteit Amsterdam 2) University of Amsterdam

With the recent advent of femtosecond time-resolved X-ray crystallography, key questions arise whether the structural dynamics of biological photoreceptors resolved by this technique represent native mechanisms. Here, we present a femtosecond to millisecond time-resolved UV-vis and mid-IR study of Photoactive Yellow Protein (PYP) in solution and crystalline forms. We observed significant differences in the PYP photodynamics under these two conditions, with a lifetime of the primary isomerized product I_0 of 0.3 ns in the crystalline phase vs. 1.5 ns in solution. Strikingly, a distinct photoproduct with a lifetime of 14 ns that was observed in the crystalline phase was not formed in the solution phase. Time-resolved mid-IR spectroscopy showed distinct transient hydrogen-bond patterns involving the carbonyl of the p-coumaric acid chromophore dependent on crystalline or solution phase. Comparing the structural events in the photocycle of crystalline and solution PYP, our results clearly demonstrate the perturbative nature of the crystal environment on the PYP photocycle. Thus, one must exercise caution when inferring native dynamical behavior across differing physical states.

> IL374. Invited Lecture
Symposium PCHEM-10 Femtobiology (Dongping Zong)

ULTRAFAST PHOTOISOMERIZATION AND ROLE OF PROTEIN ENVIRONMENT IN RHODOPSINS

Authors: Hideki Kandori[1]
Presenting Author: Hideki Kandori
1) Nagoya Institute of Technology

Biological systems utilize light as the source of signal and energy, as seen in our vision and plants' photosynthesis, respectively. Photochemical reactions of chromophore molecules in photoreceptive proteins initiate protein structural changes for various functions, whose mechanisms are of our particular interest. We have applied ultrafast spectroscopy to animal and microbial rhodopsins, and molecular mechanisms of ultrafast and highly efficient retinal photoisomerization have been studied by low-temperature FTIR spectroscopy. Role of protein environment to facilitate such specific photoreactions will be discussed.

> IL373. Invited Lecture
Symposium PCHEM-10 Femtobiology (Dongping Zong)

PICOSECOND TO MILLISECOND DYNAMICS IN THE PROTOTYPICAL REVERSIBLE PHOTOSWITCHABLE PROTEIN DRONPA

Authors: Sergey P. Laptenok[1], Agnieszka A. Gil[1], Christopher R. Hall[1,5], Andras Lukacs[3], James N. Iuliano[2], Garth A. Jones[1], Gregory M. Greetham[4], Paul Donaldson[4], Peter J. Tonge[2] and Stephen Meech[1]*
Presenting Author: Stephen Meech
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The reversibly switchable GFPs (rsGFPs) play a critical role in super-resolution microscopies such as PALM, RESOLFT among others. In these applications, some control over the rate of photoswitching is very desirable, which will require a microscopic picture of the switching mechanism. Structural studies of the light and dark adapted states of dronpa, the prototypical rsGFP, show that off to on switching involves a trans to cis isomerization, a deprotonation and a substantial reorganization of protein residues around the chromophore. In this work we combine femtosecond to millisecond time resolved infra-red experiments with isotope labelling to probe the details of the mechanism.[1]

The time resolved IR difference spectra reveal complex multiphase dynamics, which includes excited state relaxation on the picosecond timescale which is followed by some slower ground state relaxation. Surprisingly a feature that can be associated with the cis isomer does not become apparent until after nearly 100 ns has elapsed. There is then some further structural reorganization prior to the final formation of the on state following a slow (tens of microsecond) deprotonation reaction.

Reference

1. S. P. Laptenok, A. Gil, C. R. Hall, A. Lukacs, J. N. Iuliano, G. Jones, G. M. Greetham, P. Donaldson, A. Miyawaki, P. J. Tonge, and S. R. Meech '*Infrared spectroscopy reveals multistep multiple-timescale photoactivation in the photoconvertible protein archetype dronpa*', Nat. Chem. 2018, 10, 845-52

> IL370. Invited Lecture
Symposium PCHEM-10 Femtobiology (Dongping Zong)

ALLOSTERIC REGULATION OF BIOLOGICAL FUNCTION OF PHOTORECEPTOR PROTEINS

Authors: Jiali Gao[1]
Presenting Author: Jiali Gao
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Major light-harvesting complex of photosystem II (LHCII) is a photoreceptor protein that regulates energy transfer and dissipation in response to rapid fluctuations of light intensity, directly affecting the efficiency of photosynthesis. In this presentation, I will describe an investigation combining molecular dynamics simulation and temperature-jump time-resolved IR spectroscopy to understand the mechanism of energy dissipation in LHCII. I will illustrate an allosteric regulation of the global protein conformational changes induced by local conformational transitions, facilitating fluorescence quenching. In addition, I will discuss a multistate density functional theory designed to model photochemical and charge transfer processes.

> OC135. Oral Communication

Symposium PCHEM-10 Femtobiology (Dongping Zong)

MULTISCALE MODELING OF LIGHT HARVESTING IN CRYPTOPHYTE PHOTOSYNTHESIS

Authors: Carles Curutchet[1]
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1) Department of Pharmacy, Pharmaceutical Technology and Physical Chemistry & Institute of Theoretical and Computational Chemistry, University of Barcelona, Spain

The environment plays a key role in the light harvesting dynamics of photosynthetic pigment-protein complexes.[1] Specific pigment-protein interactions modulate the energy levels of the pigments, thus defining the spatial pathways of energy transfer. On the other hand, the polarizable properties of the environment screen electronic couplings between pigments, a key quantity that determines exciton delocalization and migration dynamics. Moreover, vibronic coherent features in their two-dimensional electronic spectra have been suggested to arise from the structured-nature of the spectral density of electron-phonon coupling.

Here we overview a combined QM/MM-MD computational strategy we have developed that allows exploring the impact of the environment in full atomic detail on site energies, electronic couplings and spectral densities, accounting for mutual polarization effects among the chromophores and their environment through polarizable force fields. We discuss how this strategy has allowed a better understanding on the role of the protein environment on the light harvesting properties of cryptophyte antenna complexes, which have attracted considerable attention due to their ability to display several colors and to exhibit maximal photosynthetic activity under very low-light conditions, as well as the observation of vibronic coherent features at room temperature.[2]

References

[1] (a) C. Curutchet; B. Mennucci, *Chem. Rev*. **2017**, 117, 294. (b) C. C. Jumper et al. *Curr. Opin. Chem. Biol.* **2018**, 47, 39.
[2] (a) S. J. Harrop et al. *Proc. Natl. Acad. Sci. USA* **2014**, 111, E2666. (b) C. Curutchet et al., *J. Am. Chem. Soc.* **2011**, 133, 3078. (c) M. Corbella et al. *Phys. Chem. Chem. Phys*. **2018**, 20, 21404. (d) M. Corbella et al. *ChemPhotoChem* **2019**, DOI: 10.1002/cptc.201900045

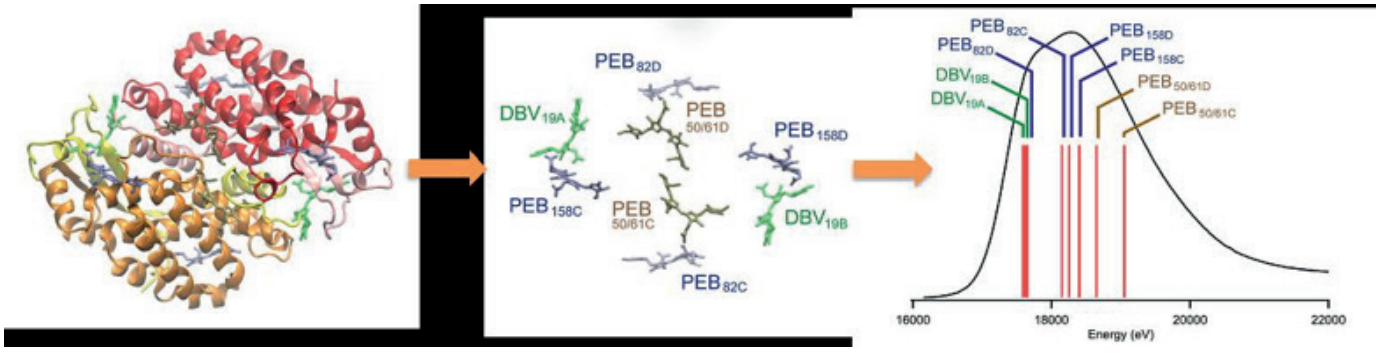

Figure 1. Structure and absorption spectrum of the phycoerythrin 545 antenna complex.

> OC136. Oral Communication
Symposium PCHEM-10 Femtobiology (Dongping Zong)

NON-STANDARD ELECTRON TRANSFER PATHWAYS IN PHOTOLYASES AND CRYPTOCHROMES

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Photolyases and cryptochromes form a vast superfamily (PCSf; phylogenetic tree in Fig. A) grouping widespread flavoproteins of similar structures but exhibiting a large variety of functions.[1] Photolyases are blue light-activated enzymes repairing UV-damaged DNA[2] and cryptochromes are mainly photoreceptors triggering diverse biological responses to light.[3] These proteins share a common mechanism of photoinduced reduction of their flavin adenine dinucleotide (FAD) cofactor, used for different purposes: activation of DNA repair for photolyases, signaling for cryptochromes (*e.g.* photomorphogenesis, entrainment of circadian clock…). In many PCSf members FAD photoreduction involves electron transfer (ET) along a chain of three conserved tryptophan residues. However, significant variations of this *standard* ET pathway are found in other PCSf members, offering the possibility to explore the diversity of solutions evolved by nature to achieve the desired function.

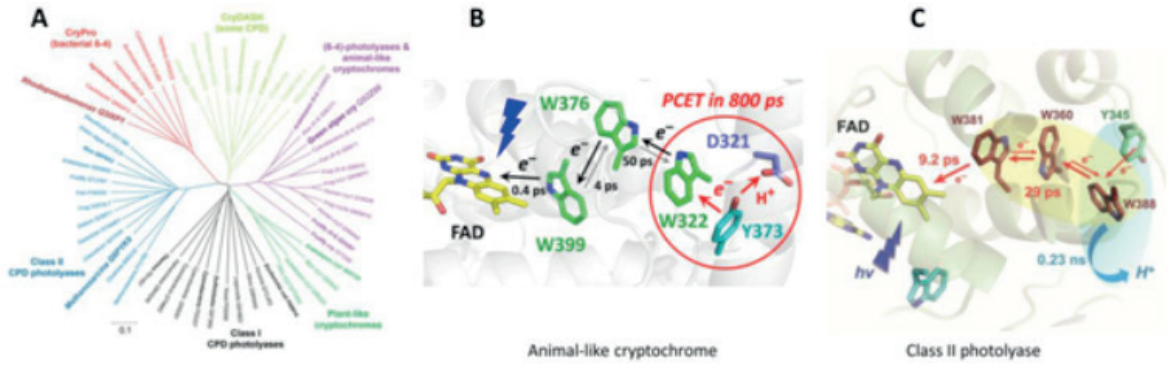

Fig. A) Phylogenetic tree of selected members of the PCSf. **B**) Exceptionally fast oxidation of the distal tyrosine by proton-coupled electron transfer in an animal-like cryptochrome. **C**) Delocalized hole transport coupled to sub-ns tryptophanyl deprotonation promotes photoreduction of a class II photolyases.[5]

We will present the detailed mechanism FAD photoreduction of a few non-standard PCSf members as studied by a combination of time-resolved transient absorption spectroscopy techniques, from hundreds of femtoseconds to seconds. An animal (6-4) photolyase will illustrate the case of an ET chain counting an additional tryptophan residue to the standard triad, further extending charge separation.[4] An animal-like cryptochrome will show the replacement of this fourth tryptophan by a tyrosine; the exceptionally fast oxidation of the distal tyrosine by proton-coupled electron transfer in ~800 ps is, about 40 times faster than the archetypal tyrosine-Z oxidation in photosystem II (Fig. B). Finally a class II CDP photolyase, will demonstrate the involvement of a completely different, non-standard, tryptophan triad, giving rise to an unusually fast deprotonation of the distal tryptophanyl radical (Fig. C), three orders of magnitude faster than in other photolyases.[5]

Continued

References
1. A. Sancar, *Chem. Rev.*, 2003, **103**, 2203.
2. K. Brettel, *et al.*, *Curr. Opin. Struct. Biol.*, 2010, **20**, 693.
3 .I. Chaves, *et al.*, *Annu. Rev. Plant Biol.*, 2011, **62**, 335.
4. R. Martin, *et al.*, *Phys. Chem. Chem. Phys.*, 2017, **19**, 24493.
5. F. Lacombat, *et al.*, *Phys. Chem. Chem. Phys.*, 2018, **20**, 25446.

> OC137. Oral Communication
Symposium PCHEM-10 Femtobiology (Dongping Zong)

FIRST STUDY OF THE PHOTODYNAMICS OF A HYDROZOAN PHOTO-SWITCHABLE FLUORESCENT PROTEIN: EXISTENCE OF DIFFERENT SWITCHING MECHANISMS.

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Recently, reversibly photoswitchable fluorescent proteins (RSFPs) have been widely applied in super-resolved fluorescence microscopy, such as reversible saturable optical fluorescence transition (RESOLFT), a super-resolved microscopy technique that allows for a significant reduction in the illumination intensities and in photobleaching. Even though photo-physical parameters (switching and fluorescence quantum yields) are linked to the resolution and image acquisition speed, the switching mechanism that controls these parameters is still a matter of debate. The most studied RSFP is Dronpa, a negative RSFP from Anthozoa (e.g. corals). The majority of studies have focused on the switching dynamics from the non-fluorescent (off) to the fluorescent (on) state because of its significant switching quantum yield of ≈ 10% (on the contrary, on-to-off switching quantum yield is only ≈ 1%). It has been reported that a *trans*-to-*cis* isomerization occurs within a few picoseconds in the excited state, followed by chromophore deprotonation in the ground state on the microsecond time scale (Warren et al. Nat. Comm. 2013, Yadav et al. J. Phys. Chem. B 2015). On the contrary, a recent report suggested that protein cage rearrangements play an important role for the isomerization process and provided evidence for both isomerization and deprotonation occurring in the ground state (Laptenok et al. Nat. Chem 2018). Here, we share results on another RSFP, rsEGFP2, an RSFP from Hydrozoa (e.g. jellyfish) which is the most common protein used in RESOLFT (Grotjohann et al. elife 2012). Time-resolved serial femtosecond crystallography (TR-SFX) at an X-ray free electron laser, combined with UV-visible transient absorption spectroscopy, showed the existence of a twisted chromophore configuration on the picosecond time-scale, with the two cyclic moieties perpendicular to each other and a dynamically restricted by the close proximity to the V151 side chain (Coquelle et al. Nat. Chem 2018). Accordingly, mutation of the latter to alanine doubles the off-to-on switching quantum yield (Coquelle et al. Nat. Chem 2018).

Using electronic and vibrational time-resolved transient absorption spectroscopy from the femtosecond to the millisecond time scale we studied the mechanism of off-to-on photoswitching in WT and mutant rsEGFP2 with various off-to-on switching quantum yields. We found that different off states and different isomerization mechanisms for the *trans*-to-*cis* isomerization can explain the variation in switching quantum yields. For example, the increase of off-to-on switching quantum yield for V151A mutant is rationalized by a sub picosecond isomerization without any intermediate in the excited state. We also characterized a 100-picosecond intermediate for the V151A mutant in the ground state that does not exist in the WT protein. We will discuss how the protein cage controls the off-to-on dynamics and the isomerization and deprotonation mechanisms in rsEGFP2.

> OC138. Oral Communication
Symposium PCHEM-10 Femtobiology (Dongping Zong)

ULTRAFAST LIGHT-INDUCED ELECTRON TRANSFER PROCESSES IN FLAVOENZYMES: RADICAL PRODUCT STATES AND PROTEIN FLEXIBILITY

Authors: Lipsa Nag[1], Jean-Christophe Lambry[1], Hannu Myllykallio[1], Ursula Liebl[1], Marten Vos[1]
Presenting Author: Marten Vos
1) Ecole Polytechnique

Flavoproteins are ubiquitous and involved in many biological functions that exploit the ability of flavins to act as electron- and/or proton-transfer intermediates. Most flavoproteins are not naturally photoactive, yet some are involved in photobiological processes. Flavin chromophores are highly fluorescent in solution, but this fluorescence can be strongly quenched in a protein environment, in particular by photoreduction or photooxidation processes. Flavin photoreduction can be achieved most importantly by electron transfer from nearby tryptophan (TrpH) or tyrosine (TyrOH) amino acids or by substrate molecules in the active site. TrpH°, Trp° and TyrO° radical forms have long been known to play functional roles as intermediates, including in the DNA photolyase/cryptochrome photoreceptor family. These species absorb in the visible and model solution spectra from pulsed radiolysis are available. This situation is different for the putative TyrOH°, state that is probably highly unstable (pK ca 2). Using ultrafast fluorescence and absorption spectroscopy of variants of the bacterial RNA methyltransferase TrmFO, we have recently proposed that this state can be formed with a lifetime of a few picoseconds, and determined its spectral signature[1]

This implies that the oxidation of TyrOH does not necessarily induce its concerted deprotonation.

We have now investigated whether the TyrOH° state is formed in other flavoproteins. This includes glucose oxidase, a well-studied model flavoprotein that harbours both tryptophan and tyrosine residues in the flavin vicinity, but where the identity and evolution of the photoproducts as identified by time-resolved visible absorption spectroscopy remained unclear[2]

Our results indicate that the now-identified TyrOH°, state also plays a role and propose a sequence of photoproducts in this enzyme.

The kinetics of electron transfer between tyrosine and flavin can be used as a probe for protein flexibility[3]

We have used this approach to study the active site of the TrmFO enzyme, that can bind three different large substrate molecules and that is known to contain flexible loops. These experiments are modeled by molecular dynamics simulations where instantaneous rates of electron transfer are evaluated along the trajectories at different temperatures.

Finally, in photoenzymes, catalysis can be initiated by charge transfer interactions between chromophore and substrate. We have started to investigate the picosecond photoreduction of flavin in fatty acid photodecarboxylase[4], where any aromatic amino acids that might compete with this reaction are located far from the flavin.

References

(1) Nag, L.; Sournia, P.; Myllykallio, H.; Liebl, U.; Vos, M. H. *J. Am. Chem. Soc.* **2017**, *139*, 11500-11505.
(2) Lukacs, A.; Zhao, R.-K.; Haigney, A.; Brust, R.; Greetham, G. M.; Towrie, M.; Tonge, P. J.; Meech, S. R. *J. Phys. Chem. B* **2012**, *116*, 5810-5818.
(3) Laptenok, S. P.; Bouzhir-Sima, L.; Lambry, J.-C.; Myllykallio, H.; Liebl, U.; Vos, M. H. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 8924-8929.
(4) Sorigué, D.; Légeret, B.; Cuiné, S.; Blangy, S.; Moulin, S.; Billon, E.; Richaud, P.; Brugière, S.; Couté, Y.; Nurizzo, D.; Müller, P.; Brettel, K.; Pignol, D.; Arnoux, P.; Li-Beisson, Y.; Peltier, G.; Beisson, F. *Science* **2017**, *357*, 903-907.

> P174. Poster

Symposium PCHEM-10 Femtobiology (Dongping Zong)

EXCITON FLUORESCENCE IN I-MOTIF DNA

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It is well known that sun light is a mutagenic agent causing various DNA damages. One of the most dangerous DNA changes are so-called cyclobutane pyrimidine dimers (CPDs), which can cause skin cancer[1]. CPDs and other photoproducts mostly appear from direct interaction of UVB solar radiation with DNA. The reaction of CPD formation proceeds very fast - within ~1 ps, implying no rearrangement of the stacked bases[2]. From which excited states the photochemical reactions start and what is the nature of the photochemical reaction pathway is of vital importance in the understanding of the fundamental principles of DNA photochemistry. These primary photoprocesses occur on a femtosecond time scale and greatly affect the subsequent photochemistry. They have been the subjects of intense research interest during the past decade[3].

The emission dynamics on the femtosecond time scale for the neutral single-stranded and hemi-protonated stacking forms of cytosine chains $(dC)_{10}$ have been studied. For the i-motif form, two components are seen in the fluorescence up-conversion decay curves acquired up to the 6 ps. The fast component can be referred to as the monomer-like emission from the locally excited state. The slow component is shifted to longer wavelengths, with the shift that correlates (by the sign and amount) with the red shift of the lowest-energy state seen in the absorption spectrum of the hemi-protonated $(dC)_{10}$. QM calculations of the excitation spectrum of a tetramer i-motif structure suggest an excitonic nature of low-energy transitions in i-motif. We attribute the slow decaying component to the delocalized (excitonic) excited state. The fraction of the bases engaged in the delocalized state is comparable with the fraction of the locally excited bases. The delocalized emissive state is probably a precursor for the further formation of long-lived charge-transfer excimer states observed in i-motif structures.

This work was supported by the RFBR, project No. 18-33-01233.

References

[1.] Brash, D. E. *et al.* A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc. Natl. Acad. Sci.* **88**, 10124–10128 (1991).
[2]. Schreier, W. J. *et al.* Thymine Dimerization in DNA Model Systems: Cyclobutane Photolesion Is Predominantly Formed via the Singlet Channel. *J. Am. Chem. Soc.* **131**, 5038–5039 (2009).
[3]. Pollum, M., Martínez-Fernández, L. & Crespo-Hernández, C. E. Photochemistry of Nucleic Acid Bases and Their Thio- and Aza-Analogues in Solution. in *Photoinduced Phenomena in Nucleic Acids I* (eds. Barbatti, M., Borin, A. C. & Ullrich, S.) **355**, 245–327 (Springer International Publishing, 2014).

> P175. Poster

Symposium PCHEM-10 Femtobiology (Dongping Zong)

EXCITED STATE DEACTIVATION MECHANISMS IN INDIRUBIN: PHOTOSTABILITY AND INTERACTION (WITH G-QUADRUPLEX)

Authors: Daniela Sarmento[1], Adelino Glavão[2], João Sérgio Seixas de Melo[1]
Presenting Author: Daniela Sarmento
1) Coimbra Chemistry Centre, University of Coimbra 2) Instituto Superior Técnico, University of Lisbon

Introduction

Interaction between aromatic molecules and nucleic acids (NA) is a subject of rising interest. G-quadruplex, guanine rich NA with structural features very different from the regular double helix, have recently gained interest as targets for anticancer drugs. [1] Indirubin (INR) is one of the structural isomers of indigo. Its relevance is linked to its use, known for millennia, in traditional Chinese medicine, as one of the components of *Danggui Longhui Wan,* a medicine for the treatment of Leukemia.[2] In Indigo, as with INR, the excited state deactivation is known to be dominated by radiationless processes (with the internal conversion quantum yield >99.9%).[3] In indigo the mechanism is associated to a fast intramolecular (single) proton transfer (ESPT).[4]

Methods

INR was investigated by both steady-state and transient techniques (fs-TA), together with TDDFT calculations aiming to further understand the mechanism behind this extremely efficient non-radiative process.

Results

Comparing to indigo, INR shows a more efficient radiationless deactivation and consequently a high stability towards light. Whereas the highly efficient dark deactivation process in indigo is linked to a single ESPT, with INR in non-viscous solvents, an additional pathway exists involving rotation between the two indole-like moieties. This leads to a *syn*-conformer with a more efficient radiationless deactivation pathway. The rotation is absent in glycerol leading to an increase of the fluorescence quantum deactivation pathway (Fig.1).

Preliminary TDDFT results suggests that interaction between INR and G-Quadruplex is favorable involving hydrogen bonding between the carbonyl group of INR and amine group of guanine.

Conclusion

The excited state characterization of INR shows that the internal radiationless deactivation pathway is the key for the stability of this molecule. ESPT and isomerization in the excited state are associated with the mechanism of this photostability. The interaction studies with G-Quadruplex can be monitored by steady-state and fast kinetic (fs-TA and ps) data following the changes on the photophysics of INR.

We acknowledge funding by FEDER-COMPETE.Coimbra Chemistry Centre is supported by the Fundação para a Ciência e a Tecnologia (FCT)-UID/QUI/UI0313/2013. PhD Grant SFRH/BD/140890/2018

There is no conflict interest.

References

1.Sneha Paul, Anunay Samanta, J. Phys. Chem. B, 2018, 122, 2277-2286.
2. N. Gaboriaud-Kolar et al., Expert Opin., 2010, 583-593.
3. J. S. Seixas de Melo, C. Serpa, H. D. Burrows and L. G. Arnaut, Ang. Chem. Int. Ed. Eng., 2007, 46, 2094-2096.
4. J. Pina, D. Sarmento, M. Accoto, P. L. Gentili, L. Vaccaro, A. Galvão and J. S. Seixas de Melo, J. Phys. Chem. B, **2017**, 121, 2308-2318.

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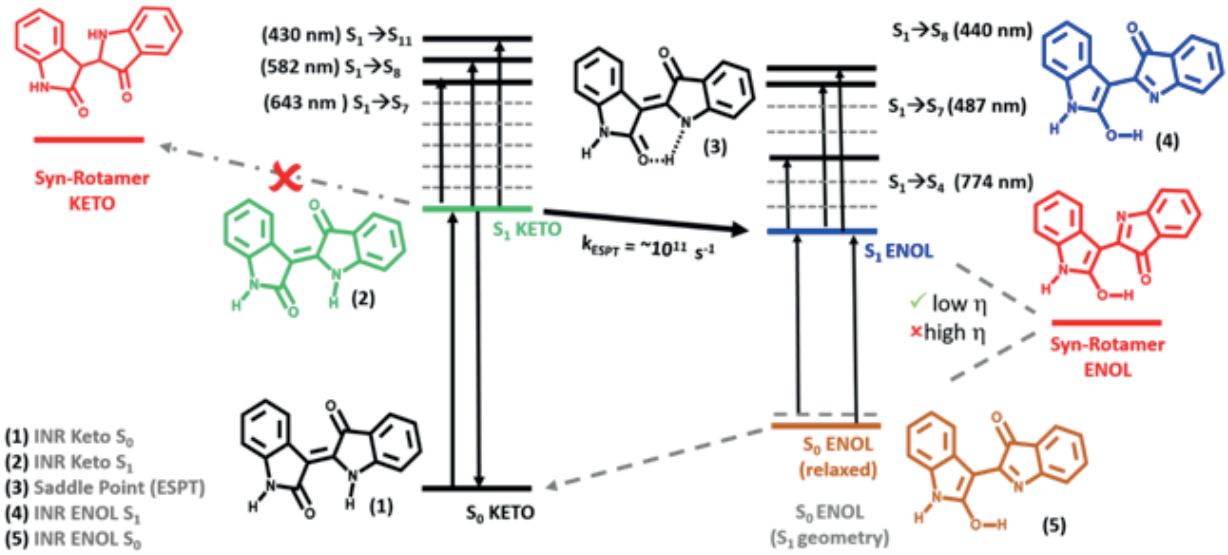

Fig.1 – Illustrative scheme for the excited state deactivation of INR.

> OC139. Oral Communication

Symposium PCHEM-11 Short Communications on Physical and Chemical Photobiology (Cristina Flors)

PHOTODAMAGE TO LYSOZYME VIA TYPE I AND TYPE II REACTIONS SENSITIZED BY EYE LENS CHROMOPHORES

Authors: Ekaterina Savina[1], Peter Sherin[1,2]
Presenting Author: Ekaterina Savina
1) International Tomography Center SB RAS 2) Novosibirsk State University

In living tissues proteins could be photodamaged via direct reactions with photoexcited chromophores (Type I reactions) or via reactions with reactive oxygen species (Type II reactions). The eye lens is a unique tissue due to (i) very low level of molecular oxygen and (ii) large absorption of UV-A light (315-400 nm) by endogenous chromophores, kynurenine and its derivatives. This could result in significant photodamage to eye lens proteins via type I reactions sensitized by kynurenines. However, under ambient conditions the concentrations of photogenerated radicals within the eye lens are significantly below the concentration of residual oxygen and a contribution of Type II reactions could not be excluded. To clarify the role of both type reactions, their mechanisms, dynamics and products should be studied in details.

The main purpose of this work is to evaluate the Photodamage to a model protein lysozyme via Type I or Type II reactions sensitized by kynurenic acid (KNA), one of the most effective triplet state generator among kynurenines. Experiments were carried out under conditions of equal light absorption in both types of photolysis. The work was performed with the use of steady-state and time-resolved optical methods, gel electrophoresis, high-performance liquid chromatography with mass spectrometry detection (HPLC-MS).

Type I photolysis was realized by the use of pulsed laser radiation to form high concentrations of radicals; Type II photolysis – by continuous-wave radiation from lamp to generate low concentrations of KNA radicals, which were effectively intercepted by residual oxygen with the formation of superoxide anion. In the case of Type I photolysis, rapid degradation of monomeric protein is followed by its cross-linking to dimeric and trimeric forms. Other modifications include the oxygen atom transfer to lysozyme from KNA with formation of deoxygenated KNA products and covalent binding of KNA to lysozyme. During the Type II photolysis only small decomposition of lysozyme was observed with minor presence of dimeric forms. This indicates efficient recombination of lysozyme radicals and superoxide with the restoration of initial reagents. The obtained results clearly show that Type I reactions provide significantly larger impact on the protein integrity and they may play an important role in lens protein modifications during normal aging and development of cataracts.

This work was supported by Russian ScienceFoundation (project № 18-73-10014).

> OC140. Oral Communication

Symposium PCHEM-11 Short Communications on Physical and Chemical Photobiology (Cristina Flors)

THE INTERACTION BETWEEN BISCARBOCYANINE DYE AND AROMATIC AMINO ACIDS IN ALBUMIN IS ESSENTIAL FOR SUPEROXIDE ANION RADICAL FORMATION IN PHOTOSENSITIZATION

Authors: Alexandra Radchenko[1], Alexey Kostuykov[1], Mikhail Mestergazi[2], Ioury Borissevitch[3, 4], Vladimir Kuzmin[1]
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Cyanine dyes are widely used in biology and medicine for fluorescent labeling of molecules and tissue contrast. Bicarbocyanine dyes are promising photosensitizers for photodynamic therapy (PDT) characterized by high efficiency of intercombination conversion [1, 2]. The efficiency of PDT depends on the amount and type of reactive oxygen species generated by a photosensitizer. In this paper, we present the results of a study of the interaction of 2,6-bis-(3,7-di-N-ethyl-benzo[1,2-d:4,3-d`]bistiazol-)-[N-methyl-3,3`-dimethyl-indocarbocyanine] perchlorate (BICC) with aromatic amino acids in human serum albumin (HSA) as the basis for the generation of the superoxide anion radical.

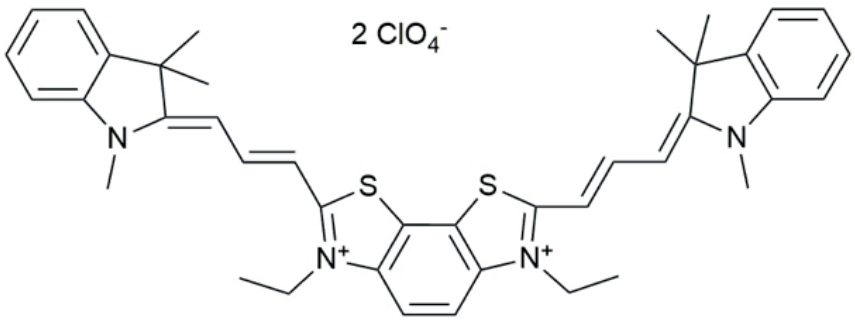
Using fluorescence and time-resolved spectroscopy, we have shown that BICC interacts and statically quenches the fluorescence of Tyr and Trp amino acids in HSA molecule. Molecular semi-flexible docking identified possible positions for BICC in HSA. In the flash photolysis experiments, the BICC triplet state accepted an electron from Tyr/Trp in HSA and formed a radical anion, which can produce a superoxide anion radical in interaction with oxygen. The superoxide anion radical was observed in colon carcinoma tumor cells HCT116 loaded with BICC upon photoactivation using confocal fluorescence microscopy.

In conclusion, the process of electron transfer from the aromatic amino acids in the albumin to the biscarbocyanine dye in the triplet state leads to the superoxide anion radical generation upon the photoactivation. This process is essential for the oxidative stress in the cell and may serve as a ground in the design of new PDT agents.

This work was supported by the Russian Science Foundation, Agreement No. 18-13-00463. Spectroscopic measurements and confocal laser scanning fluorescence microscopy were carried out in the IBCP RAS Centrum “New Materials and Technologies.” There are no conflicts to declare.

References

1. L. S. Murakami et al., Photocytotoxicity of a cyanine dye with two chromophores toward melanoma and normal cells, Biochim. Biophys. Acta., 2015, 1850, 1150-1157;
2. A. A. Kostyukov et al., Interaction of the triplet state of biscarbocyanine dye with nitroxyl radical, High Energy Chem., 2019, 53, 76-78.



> OC141. Oral Communication

Symposium PCHEM-11 Short Communications on Physical and Chemical Photobiology (Cristina Flors)

POLYMETHINE DYES AS PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

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Introduction

Extensive efforts have been devoted to the development of near-infrared (NIR) dyes for biological applications, especially for photodynamic therapy (PDT) and imaging. [1,2]

Polymethine dyes deserve to be counted among innovative potential photosensitizers (PSs) for their strong absorption in the NIR region perfectly matching the biological tissues' transparency window (600-900 nm). [3] Moreover, squaraines and cyanines possess high absorption coefficients, bright fluorescence and photostability in organic media. [4] However, in physiological conditions, their chemical instability and self-aggregation properties limit their widely applications. In this context, the incorporation of these dyes in nanoparticles (NPs) or the formation of complexes with proteins is extremely important in order to prevent the formation of dye aggregates in aqueous environment and protect the photophysical characteristics from nucleophilic attacks.

Results and Discussion

The present contribution deals with the design and synthesis of a new series of NIR absorbing polymethine dyes with different substitution groups to implement a structure-activity study and to determine the substitutions influence on the reactive oxygen species (ROS) production, cellular uptake and photodynamic activity. [5,6] These dyes were then encapsulated in solid lipid nanoparticles (SLN) to promote their use in physiological conditions.

Conclusions

In summary, the results described herein suggest that polymethine dyes can be considered promising, photosensitizers for use in photodynamic anticancer treatment. The easiness of preparation and the possibility to provide a large variety of molecules with different structural properties will allow a wide and complete SAR study. SLN-dye complexes exhibit excellent optical properties, remarkable photostability, biocompatibility and efficient cellular internalization.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest

References

[1] Abrahamse, H.; Hamblin, M. R. Biochem J. 2016, 473, 347–364
[2] Luo, S.; Zhang, E.; Su, Y.; Cheng, T.; Shi, C. Biomaterials 2011, 32, 7127–38.
[3] Avirah, R.R.; Jayaram, D.T.; Adarsh, N.; Ramaiah, D.. Org. Biomol. Chem. 2012, 10, 911–920.
[4] Barbero, N.; Magistris, C.; Park, J.; Saccone, D.; Quagliotto, P.; Buscaino, R.; Medana, C.; Barolo, C.; Viscardi, G. Org. Lett. 2015, 17, 3306-3309.
[5] Serpe, L.; Ellena, S.; Barbero, N.; Foglietta, F.; Prandini, F.; Gallo, M.P.; Levi, R.; Barolo, C.; Canaparo, R.; Visentin, S. Eur. J. Med. Chem. 2016, 113, 187-197
[6] Ciubini B., Visentin S., Serpe L., Canaparo R., Fin A., Barbero N. Dyes and Pigments 2019, 160, 806–813.

> OC142. Oral Communication

Symposium PCHEM-11 Short Communications on Physical and Chemical Photobiology (Cristina Flors)

SQUARAINES AS SENSITIVE "TURN–ON" PROBES FOR QUANTITATIVE DETECTION OF CT-DNA AND PROTEINS

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Rapid, precise and sensitive detection of proteins and DNA is critical in diagnostic fields because the over/under expression of specific proteins are associated with many diseases. Many fluorescent dyes for protein detection have been developed even if most of the fluorescent probes show some drawbacks as the lengthy procedure, small Stock Shifts and aggregation. In fact, fluorescent dyes exhibit strong emission and high quantum yield in organic solvents, but decreased intensity and quantum yields when they are in aqueous media due to "aggregation caused quenching" (ACQ).

Squaraine dyes (SQs), an interesting class of fluorescent dyes displaying intense fluorescence in the red to near infrared region (NIR), also tend to form aggregates in aqueous solution. Compared with probes of emission wavelengths in the visible range, NIR SQs could be advantageous in biological application due to lower photodamage, minimal fluorescence of background and lower light scattering. Recently we reported a thermodynamic and kinetic study of the formation of supramolecular adducts between a series of squaraine dyes and bovine serum albumin (BSA) [1]. Upon addition of the squaraine into the BSA solution, squaraine molecules aggregate and may entangle with the hydrophobic segments of the BSA chains. Actually, the fluorescence quantum yields of the SQ-BSA adducts in buffer are comparable with the ones reported in organic solvents. These results make these adducts very interesting as potential probes or photosensitizers for different applications (bioimaging, photodynamic therapy, etc.).

Inspired by our results, herein we present a study concerning the interaction between a series of SQs with different proteins and ct-DNA in order to investigate supramolecular adducts formation with Aggregation-Induced Emission (AIE) properties. Our results demonstrate that various functional groups on the SQs can affect their interaction with proteins based on their binding affinities. Since cell free circulating tumour DNA (ct-DNA) is a potential surrogate for the entire tumour genome, the quantification of ct-DNA in a liquid biopsy may help to obtain genetic follow-up data that are clinically needed [2]. Here we report preliminary data on ct-DNA quantification in human serum samples, based on AIE-active squaraine derivatives.

Acknowledgements
Authors acknowledge the financial support from the University of Torino (Ricerca Locale ex-60%, Bando 2018).

Conflict of Interest
The authors declare no conflict of interest

References

[1] N. Barbero, C. Butnarasu S. Visentin, C. Barolo *Chem. Asian. J*, **2019**, *14, 896-903*.
[2] E. Heitzer, P. Ulz, J.B. Geigle *Clinical Chemistry,* **2015**, *61,*112-113.

> OC143. Oral Communication

Symposium PCHEM-11 Short Communications on Physical and Chemical Photobiology (Cristina Flors)

DELAYED LUMINESCENCE BY AN IN VITRO MODEL FOR THE STUDY OF MECHANISM INVOLVED IN ALZHEIMER'S DISEASE

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The research and the understanding of the new mechanisms involved in the mitochondrial quality control can allow to identify new therapeutic treatments of neurodegenerative diseases involving mitochondrial dysfunction. In this context the analysis of Delayed Luminescence emitted by in vitro models for the study of Alzheimer\'s disease (AD) can give new insight of the alterations of mitochondria functional state and of the collective properties linked to the electronic transport in the mitochondrial respiratory chain.

It well known that that AD is characterized by intracellular and extracellular protein aggregates in the brain, including microtubule-associated protein tau and cleavage products of the amyloid precursor protein, beta-amyloid (Aβ). Several evidences have shown that elevated Aβ levels contribute to the mitochondrial abnormalities. Amyloid precursor protein (APP) and Aβ are found in mitochondrial membranes and interact with mitochondrial proteins. Overproduction of the APP and Aβ may affect dynamics of mitochondrial fusion/fission, impair mitochondrial transport, disrupt the electron transfer chain, increase reactive oxygen species (ROS) production, and alteration of calcium homeostasis, which are the hallmarks of mitochondrial diseases. Further, a significant reduction of the protein content of Complex I of the respiratory chain, of its activity and of energy production, characteristic signs of the reduction of energy metabolism associated to AD, were observed.

Delayed Luminescence (DL) is the phenomenon of photo-induced and ultra-weak emission of optical photons. Its temporal decay dynamics extends over time (seconds or minutes) after switching off the excitation source. The intensity is about 10^3-10^5 times lower than that of fluorescence or phosphorescence. Previous researches carried on Jurkat-T leukemic cells, follicular tumors and glioblastoma, also using substances that target the mitochondria, and in particular the process of electron transfer in Complex I, have shown how the DL is able to detect the activation of apoptotic pathways and oxidative stress.

The investigation was performed on an in vitro animal model for the study of AD by using primary cell cultures of Olfactory Ensheathing Cells (OECs), glial cells of the olfactory system and whose loss of functionality is the first marker of the AD. The cell cultures have been exposed to Aβ(1-42) native full-length peptide or to Aβ(25-35), a toxic fragment of Aβ, or Aβ(35-25), a no toxic Aβ fragment both in absence and in presence of Astaxanthin, a well-known antioxidant. The DL experiments were performed, using a dedicated equipment, on 20μl single drops of cell culture suspension.

DL intensity and kinetics changes as a function of the treatments were measured. In particular, an increase in DL emission, when compared with the untreated cells used as control, was observed when the cells were exposed to Aβ(25-35) fragment. This emission appears quenched in presence of Astaxanthin.

> OC144. Oral Communication

Symposium PCHEM-11 Short Communications on Physical and Chemical Photobiology (Cristina Flors)

PHOTOACTIVE NANOPARTICLES AS SWITCHABLE ULTRA-BRIGHT LABELS OF EUKARYOTIC AND BACTERIA CELLS

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Introduction

Functional organic nanomaterials (FONs), initially restricted to the field of data display and lighting, have crossed the field of nanomedicine at a staggering rate for the last decade. Based on self-assembled hydrophilic and lipophilic structures, most of them are exclusively composed of fluorescent p-conjugated polymeric or monomeric units. They exhibit remarkable brightness thanks to the high spatial confinement of large amounts of emissive units. They have become well praised in the field of optical bioimaging up to the near infrared range to label cancer cells upon endocytosis, which represents the main encountered internalization pathway. Curiously, little attention has been paid to their fate after cellular uptake, their drug delivery ability, and their combination with complementary labels to image bacteria in addition to eukaryotic cells.

Methods

Small fluorophores, endowed with large charge transfer and hydrogen bonding ability, have been synthesized. FONs were obtained upon nanoprecipitation in water of concentrated solutions of fluorophores. Photochromes serving as drug models and doxorubicin, an anticancerous agent, were encapsulated during the nanoprecipitation process. All cell assays were conducted on mesothelioma (meso 11, 13), lung (ADCA 117) cancer cell lines. Extension toward the labelling of bacteria (*S. aureus* and *E coli*) was also performed. Fluorescence confocal microscopy, electron microscopy, were used to investigate FON interactions and long-term fate after cell internalization.

Results and Discussion

Ultra-bright FONs were obtained and showed high cellular uptake following a clear endocytosis pathway.[1] After combination with specifically designed photochromes, their emission signal could quantitatively be quenched. Progressive recouvery of fluorescence was observed during cell uptake, which proved erosion into individual units upon interactions with the enzymatic and chemical surroundings. Such erosion was harnessed to release drugs, showing a one-day delivery delay compared to drugs in solution.[2] The high payload of photoactive units also imparts FONs with a large surface density of functional units, leading to strong interactions with pathogen cell walls and highly sensitive detection.[3]

Conclusion

FON versatility arises from the infinite synthetic possibilities permitted by organic chemistry involving fluorophores with varying functionality and multivalency. FON dissociation upon interactions with the lipid membrane of the endosomal/lysosomal compartments in eukaryotic cells stems from their non-covalent and hydrophobic structure. By contrast, FONs with a strong hydrogen-bonding character leads to extensive interactions with the outer bacteria cell walls, easily detectable by fluorescence microscopy.

References

1. C. Linot et al, ACS Appl. Mater. Interfaces, 9:14242-14257, 2017.
2. J. Boucard et al, Small, 14:1802307, 2018.
3. J. Boucard et al, ACS Omega, 3: 17392–17402, 2018.

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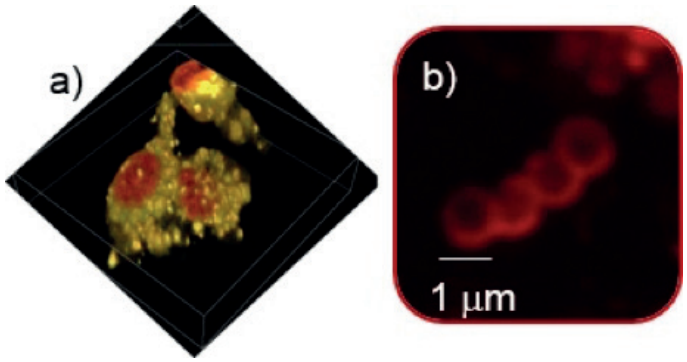

Figure 1. a) Meso cancer cells treated with doxorubicin-doped FONs. b) Confocal fluorescence microscopy of FON after 5 min incubation with S. aureus.

> OC145. Oral Communication

Symposium PCHEM-11 Short Communications on Physical and Chemical Photobiology (Cristina Flors)

NANOSTRUCTURES FOR SINGLET OXYGEN GENERATION FROM DIATOMS MICROALGAE

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Photodynamic therapy (PDT) allows non-invasive light-actuated treatment for skin/inner cancers and other tissue impairments. PDT is based on the uptake of photosensitive molecules (photosensitizers, PSs), by damaged or cancer cells.[1] After irradiation, PSs promote an electron transfer which generates singlet oxygen species (SOs), causing direct tissue necrosis. Even though a wide range of organic photosensitizers has been explored so far, the major issue observed for most PSs is related to their low stability and solubility in aqueous media. To overcome this limit, photosensitizers can be stabilized by confinement into porous microstructures or nanoparticles. Mesoporous silica represents a convenient substrate for PSs [2], even if its production needs toxic silicon precursors and energy/time consuming processes, usually associated with high temperature, high pressure and strong acidity. In this framework, we investigated diatoms microalgae as natural biofactories of mesoporous silica shells available in large scale with mild conditions.[3] We fed *Thalassiosira weissflogii* diatoms with a fluorene-based photosensitizer bearing a triethoxysilyl moiety. The *in vivo* incorporation experiments of the fluorene based PS were performed after collecting cells (1100 rpm, 15 min). The PS solution in DMSO:diatoms medium was added to cells till 1 mM final concentration. The PS incorporation before and after biosilica isolation was monitored by bidimensional fluorescence microscopy, confocal microscopy, FT-IR and SEM-EDX spectroscopies. After extraction, the functionalized biosilica was proven to enhance the singlet oxygen generation both in apolar and polar solvents. The singlet oxygen (SO) production of the PS functionalized biosilica was evaluated in toluene (phosphorescence at 1274 nm under dye excitation); SO generation was also observed under 1-photon and 2-photon excitation of the PS in polar solvents, using 1,3-diphenylisobenzofuran (DPBF) and Sensor Green probes.

Our results show that contrary to the molecular PS ability to generate singlet oxygen only in apolar solvents, the diatoms biosilica intimately embedding PS can generate singlet oxigen also in water and polar solvents such as ethanol and methanol. Our route represents a promising way to develop highly efficient materials for PDT based therapy.

Acknowledgements
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References

1.M. Sivasubramanian, Y.C. Chuang, Molecules, 24(3), doi: 10.3390/molecules24030520 (2019)
2. M. Gary-Bobo, Y. Mir, C. Rouxel, D. Brevet, I. Basile, M. Maynadier, O. Vaillant, O. Mongin, M. Blanchard-Desce, A. Morère, M. Garcia, J. O. Durand, L. Raehm, Angew. Chem. Int. Ed. 50, 11425–11429 (2011)
3. R. Ragni, S. R. Cicco, D. Vona, G. M. Farinola, Adv. Mater. 30(19), No. 1704289 (2018)

> OC146. Oral Communication

Symposium PCHEM-11 Short Communications on Physical and Chemical Photobiology (Cristina Flors)

LIGHT NANOSCOPY FOR LABELLED AND NATURALLY FLUORESCENT SYSTEMS

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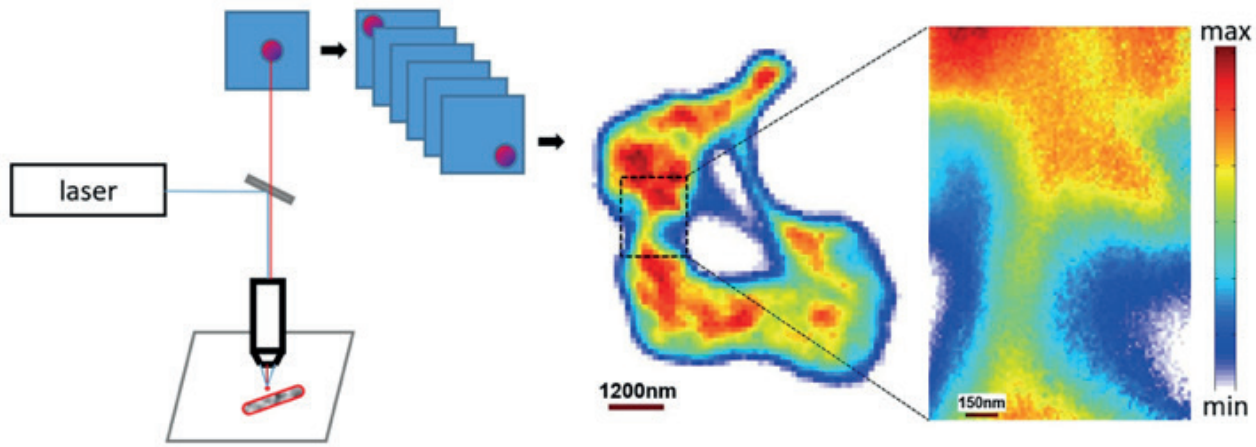
During the last two decades many methods were developed to overcome the diffraction limit of classical optical microscopy which for green excitation light and a standard objective of 1-1.45 numerical aperture is around 170-250 nm. For this reason, diffraction limited microscopy does not give access to nanoscopic scale, however super-resolution techniques overcome this barrier. Most of these techniques are based on the use of random activation and localization of fluorophores (PALM, STORM), the difference between spontaneous and stimulated emission (STED), or the patterned sample illumination (SIM). Nevertheless, these techniques are limited either by the requirement of special photo-switchable or high powers withstanding fluorophores, or non-trivial computational methods to achieve the final super-resolution image. Moreover, most of them are relative expensive and complex, and for the best results a combination of few methodologies must be used [1].

As a solution, a relatively simple and cheap super-resolution imaging technique was developed in our laboratory. It is a laser scanning nanoscopy based on the fact that the energy distribution of a perfectly focused laser beam on a fluorescing sample is inhomogeneous. Thus, any small displacement of the sample, even smaller than the diffraction limit, gives us different illumination. After the sample is scanned with X&Y steps of ca. 10-50 nm, an image with the resolution superior to 100 nm is reconstructed from one pixel from each of the recorded images. This method enables us to study labelled compartments in various cells, and even naturally fluorescent systems *in vivo*. In this work our novel light nanoscopy is applied for naturally fluorescing photosynthetic organisms (plants, algae, cyanobacteria) and various labelled bacteria cells. Also, Z-plane scanning with 3D reconstruction of labelled and naturally fluorescent photosynthetic systems is achieved. In the figure you can see simplified scheme of our light nanoscopy method and an example image of chlorophyll autofluorescence excited with 488 nm laser light in green algae *Chlamydomonas r.* cell imaged *in vivo*.

Reference
[1] Danial J.H.S. et al, *Advanced fluorescence microscopy techniques for the life sciences*, GCSP 2016:16



> OC147. Oral Communication

Symposium PCHEM-11 Short Communications on Physical and Chemical Photobiology (Cristina Flors)

SHEDDING LIGHT ON THE SUBTLE E222Q MUTATION THAT RELIEVES THE PHOTOSWITCHING BEHAVIOUR OF FLUORESCENT PROTEINS

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Reversibly photoswitchable fluorescent proteins (RSFPs) admirably combine the genetic encoding of fluorescence with the ability to repeatedly toggle between a bright and dark state, adding a new temporal dimension to the fluorescence signal. Accordingly, in the last years RSFPs have paved the way to novel applications in cell imaging that rely on their reversible photoswitching, including many super-resolution techniques such as F-PALM, RESOLFT, and SOFI that provide nanoscale pictures of the living matter. Yet many RSFPs have been engineered by a rational approach only to a limited extent, in absence of clear structure-property relationships that in most cases make anecdotic the emergence of the photoswitching. We recently reported [Bizzarri et al. *J. Am Chem Soc.***2010**, 102, 85] how the E222Q replacement is a *single photoswitching* mutation, since it restores the intrinsic *cis-trans*photoisomerization properties of the chromophore in otherwise non-switchable *Aequorea* proteins of different color and mutation pattern (Q-RSFPs). Next, we investigated the subtle role of Q222 on the excited state photophysics of the two simplest Q-RSFPs by a combined experimental and theoretical approach, using their non-switchable ancestor EGFP as benchmark [Storti et al. *ACS Chem. Biol.* **2018**, 13(8), 2082]. Our findings link indissolubly photoswitching and Q222 presence, by a simple yet elegant scenario: largely twisted chromophore structures around the double bond (including *hula-twist* configurations) are uniquely stabilized by Q222 via H-bonds. Likely, these H-bonds subtly modulate the electronic properties of the chromophore, enabling the conical intersection that connects the excited *cis*to ground *trans*chromophore. Thus Q222 belongs to a restricted family of single mutations that change dramatically the functional phenotype of a protein. The capability to distinguish quantitatively T65S/E222Q EGFP (”WildQ”, wQ) from the spectrally identical EGFP by quantitative Optical Lock-In Detection (qOLID) witnesses the relevance of this mutation for cell imaging. Other possible applications of these derivatives will be described.

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