20th Congress of the European Society for Photobiology

August 27 – 31 2023, Lyon, France

Book of abstracts

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Program Outline

time	Sunday August 27	Monday August 28	Tuesday August 29	Wednesday August 30	Thursday August 31
08:00 - 08:30				Young investigator Awards	
08:30 - 09:00		information keynote	keynote	3½h sesssion (part 1)	keynote
09:00 - 09:30		3h15 sesssion (part 1)	keynote		keynote
09:30 - 10:00			3h sesssion (part 1)		3h sesssion (part 1)
10:00 - 10:30					
10:30 - 11:00				coffee break	
11:00 - 11:30		coffee break	coffee break	3½h sesssion (part 2)	coffee break
11:30 - 12:00		3h15 sesssion (part 2)	3h sesssion (part 2)		3h sesssion (part 2)
12:00 - 12:30					
12:30 - 13:00				Lunch	
13:00 - 13:30		Lunch	Lunch + Posters + EU projects (30 min) + Springer (90 min)		Lunch
13:30 - 14:00				3h sesssion (part 1)	
14:00 - 14:30		keynote			keynote
14:30 - 15:00		keynote			3h sesssion (part 1)
15:00 - 15:30		3½h sesssion (part 1)		coffee break	
15:30 - 16:00			3h sesssion (part 1)	3h sesssion (part 2)	
16:00 - 16:30	registration				coffee break
16:30 - 17:00		coffee break			3h sesssion (part 2)
17:00 - 17:30		3½h sesssion (part 2)	coffee break	ESP general assembly	
17:30 - 18:00			3h sesssion (part 2)		
18:00 - 18:30					
18:30 - 19:00					
19:00 - 19:30	Welcome reception			boat bording	
	inauguration			gala diner	
	keynote				

Keynote lectures

Sunday August 27

KL1 Silvia Braslavsky (Mülheim an der Ruhr, Germany)

Photoscience as a Bridge to Peace

Monday August 28

KL2 María Gabriela Lagorio (Buenos Aires, Argentina)

Fluorescence of living plants. From space to cells

KL3 Janet Bornman (Perth, Australia)

ESP, a life dedicated to all things photo and biological

KL4 Michel Sliwa (Lille, France)

The synergy of time-resolved optical spectroscopy and crystallography to reveal the mechanism of photoswitchable proteins.

Tuesday August 29

KL5 Yong Zhang (Hong Kong, China)

Light-emitting Materials and Devices for Wireless Phototherapy

KL6 Gareth I. Jenkins (Glasgow, UK)

UVR8 photoreceptor action in plant responses to UV-B radiation

Wednesday August 30

KL7 Roger Bresoli-Obach (Barcelona, Spain); ESP young investigator award 2023

Each photon counts: Singlet oxygen detection from homo- to heterogeneous samples

KL8 ESP Nadja A. Simeth-Crespi (Göttingen, Germany); ESP young investigator award 2023

Probing Biology with Photochemistry using Opto-Bioorganic Building Blocks

Thursday August 31

KL9 Shelley Minteer (Salt Lake City, USA)

Using Synthetic Biology Tools for Efficient Ammonia Production from Cyanobacteria

KL10 Jean-Luc Coll (Grenoble, France)

NIR/SWIR imaging in vivo using optically active nanotheranostic systems

KL11 Michael Hamblin (Johannesburg, South Africa)

Potentiation of antimicrobial photodynamic inactivation by inorganic salts

Photosciences as a Bridge for Peace

Silvia E. Braslavsky

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A major reason for human conflicts and colonial behavior has always been the voracity for natural resources, from food and wood to silver and gold, from slaves to work in the sugar, cacao, and cotton plantations to oil, gas, uranium and lately Litium, to satisfy the everincreasing energy hunger in the present time, all this producing nowadays the climate catastrophe, a widespread pauperization, and the consequent migration waves we are witnessing. Although the solutions are obviously political and should be found in international well-controlled agreements, scientists may contribute in several ways. Our field of research, based on the observation of natural energy-transforming processes triggered by light, such as photosynthesis with O_2 and in the absence of O_2 (bacteriorhodopsin in Archaea), as well as light sensing and even UV-light-induced repair, has reached a mature stage. Examples are the development of highly efficient photovoltaic units as well as the increasingly cheaper and more efficient ways for the use of natural light to split water and produce H_2, used as fuel or, in turn, reacting with captured environmental CO_2 for its reduction to synthetic fuels or high-value chemicals. Other photo-developments include the use of light of various wavelengths to degrade pollutants of biological and/or industrial origin and, of course, the use of light for the treatment and/or the diagnosis of diseases. There is even a recent discovery of a novel photoinduced enzyme that converts fatty acids into fuellike hydrocarbons. Several of these developments allow a non-concentrated (in a few hands) access to energy resources.

We can say that the very fast development of the knowledge and the possible applications of the interaction of light with matter in general, in particular with the biosphere, has taken place especially after the second world war. One of the major motors for the fast increase has been the very strong international collaborations exercised in very different ways, either by bilateral agreements between laboratories or Universities or between Nations, or through the several international institutions, such as the UN, UNESCO, The World Academy of Sciences (TWAS) and also strongly through the participation in and of the large international learned Societies, such as IUPAC, IUPB, IUPAB, the International Meteorological Association - to name just a few – and, in general, the ICSU (International Council of Scientific Unions, today called the International Council for Sciences, ICS) and many others. These scientific interactions and collaborations were very strong and effective even in very difficult moments as it was the cold war (which was not only cold if the various substitute wars are considered) since the ‘50s to the ’80s of last century. The present difficult times characterized by the climate catastrophe, the war in Ukraine, the COVID 19 pandemic and its consequences, plus the ever present strong consequences of the colonial times in many parts of the world (Latin-America, Africa, South-West Asia, etc.) should impulse us to increase the collaborations with all scientists of the world - independently of their political or otherwise positioning - for the development of sources of energy (and other developments) that could make all our societies free from strong unilateral international dependencies and possible wars to solve them. At the same time, I consider essential to exercise more inclusive practices in our scientific communities, not only as a way of building peace bridges through all sectors of our societies, but also to have all creative individuals participating in the process, everyone with a different approach!

Fluorescence of living plants. From space to cells

María Gabriela Lagorio

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Fluorescence, the emission of light upon absorption of photons, is a fascinating phenomenon extensively studied in various scientific domains. In the context of plant biology, fluorescence has emerged as a valuable tool for investigating photosynthesis and the vitality of plants. This talk provides an overview of fluorescence in plants, with a specific focus on chlorophyll fluorescence, encompassing observations from the global scale to the cellular level.

At the macroscopic level, remote sensing techniques utilizing satellite-based sensors have revolutionized our understanding of plant health and productivity on a global scale. Satellites, such as the Orbiting Carbon Observatory-2 (OCO-2) and TanSat, detect the fluorescence signal emitted by vegetation in the red and far-red spectral regions. By quantifying this fluorescence, researchers can obtain valuable information about plant stress and carbon assimilation rates, aiding in the monitoring of ecosystem dynamics and climate change impacts.

Moving closer to the plant level, chlorophyll fluorescence serves as an invaluable probe to investigate photosynthesis non-invasively. By measuring the fluorescence emitted from chlorophyll, researchers can gain insights into several vital processes, including light harvesting, energy dissipation, and electron transport within the photosynthetic machinery. One commonly used technique to assess chlorophyll fluorescence at leaf level is the Pulse Amplitude Modulation (PAM) fluorometry. PAM fluorometers provide real-time measurements of fluorescence parameters, such as the maximum quantum yield of photosystem II (PSII) photochemistry (Fv/Fm) and the effective quantum yield of PSII (ΦPSII). These parameters offer valuable insights into the plant's photosynthetic efficiency and can serve as sensitive indicators of stress, such as nutrient deficiencies, drought, or temperature extremes. Moreover, the analyses of the rapid fluorescence transient enables the identification of specific steps in the electron transport chain where disruptions occur as a result of biotic or abiotic factors.

At the cellular level, chlorophyll fluorescence unravels the complex interplay between light absorption, energy transfer, and photochemical reactions within the chloroplasts.

Furthermore, the talk will particularly address steady-state fluorescence, which provides valuable information about the spectral distribution of the emitted light, at the chloroplast, leaf, and canopy levels. The presentation will also introduce physical correction models aimed at mitigating artifacts caused by light reabsorption (inner filter) in leaves and plant canopies. Additionally, the field methodology known as Tractor-mounted LED-induced fluorescence will be presented, showcasing how physical models allow for the retrieval of cellular-level information from data collected at a distance.

By integrating all these multi-scale observations, scientists can deepen their understanding of plant biology and contribute to more sustainable agricultural practices and ecosystem management in the face of a changing climate.

ESP, a life dedicated to all things photo and biological

Janet F. Bornman

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The history of the European Society for Photobiology (ESP) began as an idea 39 years ago in 1984. This was followed by a task group meeting the following year, and in September 1986, ESP was launched at its first Congress in Grenoble, France. The diverse and stimulating scientific community of friends and colleagues have shaped the careers and global outlook of researchers of all ages through ESP congresses, photobiology workshops, ESP Photobiology Schools, the Society journal and themed books. The path of ESP's history to date and the science during its lifetime, reflect some of the landmark environmental events and technological advances that we have addressed and researched as we face the enormous task of trying to regain a sustainable Earth. And as with many innovations, there are usually dark and light sides – the wide-ranging benefits derived from solar radiation and controversial innovations or proposals for interventions to lessen the impact we are having on the environment. Examples include nuclear technology and modifications of the solar radiation reaching the Earth in order to reduce global warming.

Paul Crutzen, the Nobel laureate, who shared the award with Sherwood Rowland and Mario Molina for their research on protecting the Earth from extreme UV radiation by identifying ozone-depleting substances, later coined the word *Anthropocene*[1] to replace the current geological epoch, the Holocene. The implications of the discovery of the stratospheric ozone hole focussed the science on how the increased amount of UV radiation could affect human health, ecosystems and food security, leading to the treaty of the Montreal Protocol for controlling the ozonedepleting substances. The science has shown many complexities[2] along the way, with climate change affecting the stratospheric ozone layer, and *vice versa*. This Keynote will intertwine some of the highlights of ESP with the photobiological research, and ponder briefly on our future prospects.

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[2] Environmental Effects Assessment Panel. 2023. Environmental effects of stratospheric ozone depletion, UV radiation, and interactions with climate change: 2022 Quadrennial Assessment. *Photochemical & Photobiological Sciences*, **22**(5), 935–1212.

The synergy of time-resolved optical spectroscopy and crystallography to reveal the mechanism of photo-switchable proteins.

Michel SLIWA

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Among photo-active biological systems, photo-switchable proteins are key elements in important biological functions such as vision, photoprotection and photosynthesis. They can also be used in different applications such as optogenetics and super-resolution bio-imaging. Their intrinsic properties of using light in an efficient way are characterized by photo-switching dynamics that takes place on a broad time-scale, ranging from hundreds of femtoseconds to a few milliseconds. It involves several excited states and ground state intermediates, and a complex interplay between the chromophore and the protein. Even though photo-physical parameters (switching / fluorescence quantum yields…) are crucial for their used in advanced bio-imaging applications, the switching mechanism that controls these parameters is still a matter of debate. Indeed, mechanistic details, in particular on the ultra-fast photochemical time scale, remain unclear. Another challenge in deducing the photo-switching mechanism is to create a uniform picture explaining both single pulse excitation experiments used in the study of ultrafast photo-dynamics, and *in vivo* continuous light irradiation condition. I will discuss here how the synergy of time resolved optical spectroscopy and serial femtosecond crystallography allows us to reveal the photo-mechanism for photo-switchable fluorescent proteins[1-5] and orange carotenoid protein[6-9]. Crucial parameters in the photo-switching dynamics were unveiled to rationally tailor the development of efficient new photo-active proteins for bio-imaging and optogenetics.

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Light-emitting Materials and Devices for Wireless Phototherapy

Yong Zhang[1]

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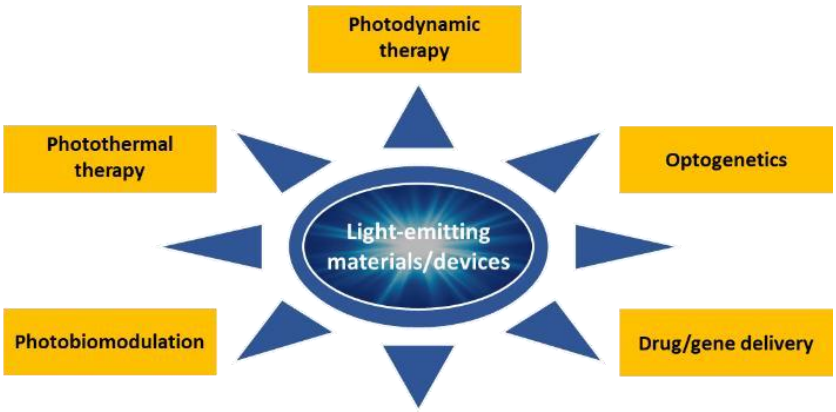
Light has proven useful in a wide range of biomedical applications such as phototherapy. Taking photodynamic therapy (PDT) as an example, it has been clinically proven effective in treating early lung, bladder, head and neck cancer and is the primary treatment for skin cancer. However, the clinical application of PDT is severely constrained by the low penetration depth of visible light through thick tissue, limiting its use to target areas only a few millimeters deep. One way to improve the range is to use nanomaterials with light-converting properties to convert deep-tissue penetrating near-infrared (NIR) light to visible light suitable for activating photosensitive drugs, extending the depth to 1cm. However, at depths beyond 1 cm, tissue remains inaccessible to light (even NIR), a critical depth limitation that renders existing phototherapy ineffective for deep-seated cancers. We have demonstrated some new treatment modalities for wireless cancer phototherapy in deep tissues using Xray activatable nanomaterials, light-emitting hydrogel implants and micro-LEDs. Use of these technologies could be extended to other light based therapeutic applications.



Light-emitting materials and devices for light based therapeutic applications

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UVR8 photoreceptor action in plant responses to UV-B radiation

Gareth I. Jenkins[1]

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Ultraviolet-B (UV-B) radiation in sunlight acts as a regulatory stimulus to plants, enabling them to acclimate to ambient conditions to optimize growth and viability. Exposure to UV-B radiation initiates extensive transcriptional reprogramming which underpins changes to metabolism, morphology and physiology. Many of these regulatory responses are mediated by UV RESISTANCE LOCUS8 (UVR8), the only plant photoreceptor known to orchestrate responses to UV-B and short-wavelength UV-A light. UVR8 exists as a homodimer in the absence of UV light. UV-B absorption by tryptophans in the primary sequence causes rapid dissociation of the dimer into monomers, which initiate signaling through interaction with other proteins. Binding of the E3 ubiquitin-ligase component CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) to UVR8 following UV-B photoreception leads to stabilization of the HY5 transcription factor, which activates many UVR8 target genes. In addition, UVR8 interacts directly with several transcription factors to regulate gene expression responses. UVR8 also binds to REPRESSOR OF UV-B PHOTOMORPHOGENESIS (RUP) proteins, which promote re-dimerization of UVR8 monomers and negatively regulate UVR8 action. However, although these UVR8 signaling partners have been identified, much still remains to be understood about their roles in UV-B responses and the mechanisms that control their interaction with UVR8.

Each photon counts: Singlet oxygen detection from homo- to hetero-geneous samples

Roger Bresolí-Obach[1],

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The investigation of the involvement of singlet oxygen ($^1O_2$) in many important processes in Biology, Materials Science, Chemistry and Medicine demands its efficient and specific detection and quantification. Due to its highly reactive nature, $^1O_2$ has only one direct method of determination, namely the detection of its phosphorescence emission at 1270 nm. Time-resolved phosphorescence detection (TRPD) of $^1O_2$ not only allows the confirmation of its presence, but also the study of its formation and decay kinetics. The determination of kinetic parameters of $^1O_2$ with time-resolved studies is indeed crucial to characterize its reactivity. In turn, these studies also provide information about the precursors of $^1O_2$. However, the phosphorescence emission of $^1O_2$ is extremely weak, thus the detection of each photon counts. Therefore, increasing the sensitivity, specificity, spatial and temporal resolution of $^1O_2$ imaging in biological systems is probably one of the major challenges in the field. During this presentation, I will discuss different possibilities to enhance our probabilities to detect $^1O_2$ such as enhancing $^1O_2$ radiative rate constant by SPR[1] or the use of highly sensitive InGaAs photodiodes.[2] Finally, $^1O_2$-selective fluorescent (nano)-probes[3] will also be discussed because they may be useful when the amounts of $^1O_2$ are very small, as the fluorescent product can accumulate, and photodetectors are very sensitive in the visible range.[4]

Acknowledgment: This research was funded by Generalitat de Catalunya (DURSI) and the European Social Fund for a predoctoral and a postdoctoral fellowship (2015FI_B 00315; 2020 BP 00066), by Fonds Wetenschappelijk Onderzoek (FWO) – Vlaanderen for a postdoctoral fellowship (12Z8120N) and by the Agencia Estatal de Investigación and FEDER for a Ramon y Cajal contract (RYC2021-032773-I)

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Probing biology with photochemistry: light-responsive labels, linkers, and building blocks

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In recent years, light has been employed as an external stimulus to photo-control diverse functional processes.[1] This approach relies on the use of small, light-responsive molecules that undergo a structural change upon irradiation, generating different functional states from a single molecule.[2] By attaching suitable substituents to such photoactuators, these molecules can be embedded in a system of choice to link their structural change to a change in the system's properties.[3] On the other hand, the sterical and electronic characteristics of the substituents influence the photophysical and photochemical properties of the core.[4] This mutual interaction needs to be finely balanced and studied in detail to rationally design probes and tools to study and modulate biological systems.

Here, we show different strategies to employ light-responsive building blocks to interact with and control biomacromolecules focusing on the 3D-structure of peptides and their supramolecular interaction. In this context, we will highlight how optimizing the substituents on different photoactuators allows us to tune several of their properties, such as their UV-Vis absorption profile and photoconversion quantum yield. We will demonstrate how these properties can be employed in various model systems. [5,6]

Eventually, we envision that deriving such design principles for an increasing number of light-responsive tools will pave the way to individually addressing a single photoactuator in a complex biologically relevant ensemble and thus, to the precise regulation of the biological machinery.

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Using Synthetic Biology Tools for Efficient Ammonia Production from Cyanobacteria

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The reduction of chemically inert nitrogen to ammonia is a critical step in the global nitrogen cycle that has utilized the Haber Bosch process over the last 100 years. With more of a focus on sustainability, then there is a focus on more sustainable alternatives to the Haber Bosch process. Microbial nitrogen fixation is a promising way to realize nitrogen reduction and ammonia production at mild conditions. This talk will discuss an engineered, non-diazotrophic Synechococcus elongatus PCC 7942 strain with nitrogen fixation activity that is constructed by integrating a modified nitrogenase gene cluster into the native genome. The engineered S. elongatus PCC 7942 strain is employed in a bioelectrochemical nitrogenfixation (e-BNF) system for ammonia production via mediated bioelectrocatalysis with methyl viologen. Because the e-BNF system supplies adequate external electrons for the turnover of nitrogenase, the nitrogen fixation activity of the engineered S. elongatus PCC 7942 strain is significantly improved. After incorporation of nitrogenase, we further used synthetic biology tools to improve the extracellular electron transfer of the cyanobacteria with the electrode through the incorporation of outer membrane cytochromes. This work may provide new insight into biological nitrogen-fixation systems and ammonium production.

NIR-I and NIR-II optically active nanosystems and their use for theranostic treatment of cancer

Jean-Luc Coll, Mans Broekgaarden, Anne-Laure Bulin, Virginie Faure, Amandine Hurbin, Véronique Josserand, Xavier Le Guevel, & Lucie Sancey

Team Cancer Targets and Experimental Therapeutics, Univ. Grenoble Alpes, INSERM U1209, CNRS UMR5309, Institute for Advanced Biosciences, 38000, Grenoble, France

Theranostic nanoparticles (TN) are a new type of nanomedical device that combine diagnostic and therapeutic capabilities for the treatment of diseases and in particular cancer. They are made from a variety of materials, including metals, polymers, and lipids, and they are designed to be safe and biocompatible. TN offer a range of advantages, such as improved drug delivery, targeted drug release, and enhanced imaging capabilities. They can be used as contrast agents to precisely detect and delineate the region to treat using MRI, X-rays, Near-infrared light or ultrasounds. But in addition, NT can be remotely and precisely activated on site using non-ionizing and/or ionizing radiations to specifically deliver therapeutic activities to the diseased cells, allowing for more effective treatments with fewer side effects.

Our team is developing multifunctional theranostic particles targeting tumor cells and/or the tumor microenvironment. We particularly focus on optical-based systems for their detection and possible on site(s) activation. Optical imaging can also be combined with ultrasonic imaging modalities and provide additional information (photoacoustics).

Our nanosystems are based on scaffolds of organic and inorganic molecules and can deliver contrast agents with different drugs or pro-drugs. Using near-infrared imaging, we can track their distribution, monitor their function and therapeutic activity using non-invasive, non-radiative, real-time in vivo imaging and then activate them once at the tumor site using light or X-Rays. These nanosystems can also be used intraoperatively for optical guided surgery of cancer.

Potentiation of Antimicrobial Photodynamic Inactivation by Inorganic Salts

Michael Hamblin 1

1 : University of Johannesburg, South Africa

One of the biggest health problems facing the world today is the inexorable rise of multi-antibiotic resistance amongst a wide range of pathogens, including Gram-positive and Gram-negative bacteria and fungi. Antimicrobial photodynamic inactivation (aPDI) uses visible/NIR excitation of a photosensitizer to produce the reactive oxygen species (ROS) singlet oxygen (Type 2) and hydroxyl radicals (Type1) that are both highly toxic to microbial cells. If the photosensitizer and the light are introduced into the infected or contaminated site the selectivity is excellent. Our laboratory has discovered that addition of simple inorganic salts can potentiate aPDI by several orders of magnitude, and may even allow oxygen-independent photoinactivation to take place. Potassium iodide is the most powerful and clinically relevant salt. Other inorganic salts such as sodium azide, potassium thiocyanate, potassium selenocyanate, potassium bromide and sodium nitrite also produce increased killing of a broad range of pathogens by up to one million times.

The underlying photochemical mechanisms will be discussed.

Parallel symposia

Monday August 28

Morning

Human antimicrobial PDT (ESP-ASP symposium)

Invited speakers:

IL1 Yanfang Feng and Tayyaba Hasan (Boston, USA)

Portable Quantum Dot Electroluminescent (QDEL) Light Sources for Point of Care Photodynamic Treatment of Deep Wound MDR Infections

IL2 Luis Arnaut (Coimbra, Portugal)

How Small Photosensitizers Achieve Big Kills in Antimicrobial Photodynamic Therapy

IL3 Yolanda Gilaberte (Zaragoza, Spain)

Antimicrobial PDT in dermatology: combination is the key

IL4 Joannna Nakonieczna (Gdansk, Poland)

Gallium porphyrin derivatives - can they be considered "antimicrobials" or are they just "disinfectants"?

Oral communications:

OC1 Francesca Giuntini: Photoantimicrobial activity of porphyrin-functionalised bioactive glass scaffolds

OC2 Yue Xiao: Targeted bactericidal activity of ruthenium complexes by NO release under irradiation

OC3 Klaudia Szymczak: Photodynamic inactivation with novel gallium metalloporphyrin: the non-antibiotic method to overcome S. aureus and P. aeruginosa biofilms

OC4 Agata Wozniak-Pawlikowska: Novel gallium photosensitizers in light-dependent action as an antivirulence strategy against the Staphylococcus aureus and Pseudomonas aeruginosa

Flash presentations (see poster sessions for abstracts):

Annette Wimmer: With blue light against biofilms: Berberine as natural photosensitizer for Photodynamic Inactivation of Gram+ and Gram- bacteria

Cristina Dias: Antimicrobial properties of marine origin materials obtained by a sustainable approach

Lucy Sinclair: Bactericidal Efficacy and Cytotoxic Responses of Pseudomonas aeruginosa to Low Irradiance 405-nm
Light

Portable Quantum Dot Electroluminescent (QDEL) Light Sources for Point of Care Photodynamic Treatment of Deep Wound MDR Infections

Yanfang Feng[1], Manuel A. Triana[2], Caroline Coradi Tonon[1], Shakir Khan[1,3], Yajie Dong[2,4], Tayyaba Hasan[1,5]

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5 Harvard University and Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

Introduction: Chronic wound infections hinder healing and strain healthcare systems (1). Antibiotics are commonly used but face challenges due to multidrug-resistant (MDR) bacteria (2). Antimicrobial photodynamic therapy (aPDT) offers the potential for treating MDR infections (3). However, current aPDT rely on expensive, non-portable lasers and hospital settings. We utilized affordable, wearable optical devices to create a user-friendly optical platform for point-of-care wound sterilization with aPDT.

Materials and Methods: This platform comprises two elements: 1) methylene blue (MB) the broadspectrum antimicrobial photosensitizer (PS) for aPDT, and 2) a lightweight, wearable quantum dot or organic light-emitting diode (QLED or OLED) illuminator that activates MB to perform aPDT (4,5). The disinfection assessments by bacteria suspension, pig skin wound infection, and mice wound infection.

Results: For bacterial suspension, at an MB concentration of 100 µM, a power source voltage of 4.5 V, and one hour of LED illumination, the aPDT platform achieved an average bacterial killing of 5 logs for the representative gram-negative MDR strain (E. coli) and 9 logs for the representative gram-positive MDR strain (MRSA). Tests on pig skin wound infections showed approximately 2 logs of bacterial inactivation for immediate infections and 1,5 - 2.5 logs for established infections in both gram-positive and gram-negative infections. In mouse models, wearing the optical aPDT device with 300 µM and 4.5 V power source voltage for 75 minutes resulted in at least 90% MDR pathogen elimination.

Conclusion: We created a portable aPDT platform that effectively treats MDR wound infections at the point of care. Its powerful bacterial killing capabilities were demonstrated through various testing models. This platform has the potential to transform clinical treatment for patients with wounds in the face of increasing MDR pathogen prevalence.

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How small photosensitizers achieve big kills in antimicrobial Photodynamic Therapy

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Many different dyes can reduce the number of colony-forming units (CFU) of planktonic bacteria by at least 3 orders of magnitude, with clinically relevant light doses, and have a bactericidal effect. However, the structural diversity of photosensitizers in clinical studies for photodynamic disinfection is surprisingly small. In particular, clinical studies of infected wounds – an important and amenable target for aPDT – have only been using 5-aminolevulinic acid derivatives, phenothiazinium dyes and a phthalocyanine [1]. A major challenge in these clinical studies is that bacteria is present in the form of biofilms.

We have shown that dicationic *meso*-imidazolyl porphyrins with low molecular weight can partition to biofilms, from aqueous solution, and infiltrate the biofilms while remaining photoactive [2]. We succeeded to photoinactive biofilms at low porphyrin concentration using light at 415 nm. However, this wavelength is not ideal for clinical translation. In this work we report the synthesis, characterization and aPDT of bacteria in planktonic and biofilm forms with the analogous chlorin and light at 650 nm. aPDT with dicationic *meso*-imidazolyl chlorin after 1 h of incubation either with *E. coli* or *S. aureus* reduces the number of CFUs by >7 log units at 1 μM and 5 J/cm^2. Adding 50 mM of KI [3], we reduced *S. aureus* biofilms CFUs by 9 log units at 1 μM and 5 J/cm^2. These conditions do not significantly reduce the viability of HaCaT cells. This dicationic chlorin was also successfully employed to photoinactivate virus. It is very a promising photosensitizer for aPDT.

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Antimicrobial photodynamic therapy in Dermatology: combination is the key

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Combination approaches of antimicrobial photodynamic therapy (aPDT) plus antibiotics or antifungals to kill bacteria and fungi *in vitro* is the key to treat skin and mucocutaneous infections. The combination can prevent failure in the fight against these microorganisms: antimicrobial drugs can increase the susceptibility of microorganisms to aPDT and prevent the possibility of regrowth of those that were not inactivated during the irradiation; meanwhile, aPDT is effective regardless of the resistance pattern of the strain and their use does not contribute to the selection of resistant.

Additive or synergistic antimicrobial effects *in vitro* are evaluated and the best combinations are presented for dermatological infections such as onychomycoses, deep fungal infections and chronic wounds, among others.

The use of combined treatment of aPDT with antimicrobials could help overcome the difficulty of fighting high level of resistance microorganisms and, as it is a multi-target approach, it could make the selection of resistant microorganisms more difficult.

Gallium porphyrin derivatives - can they be considered "antimicrobials" or are they just "disinfectants"?

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Resistance to conventional antimicrobials has become a major problem and a threat to human health and life. The number of newly discovered antibiotics is decreasing every year, and big pharmaceutical companies are not interested in introducing new antibiotics, because there is selection of resistant microorganisms within a short time after their use. For this reason, it is extremely important to develop new antimicrobial strategies whose mechanism of action differs from that of antibiotics and to which resistance does not develop so easily and quickly. Gallium(III) ions are one such agent that exhibit excellent antimicrobial activity, even against microorganisms that manifest a multidrug-resistant phenotype. The biocidal mechanism of action of gallium(III) ions is based on their molecular similarity to iron ions. However, since gallium(III) ions do not undergo redox reactions like iron, they inhibit irondependent cellular metabolic processes. This leads to the death of the bacteria. The main problem with the use of gallium(III) ions is their bioavailability.

A major problem with the use of gallium (III) ions is its bioavailability. One way to improve the bioavailability of gallium ions is to incorporate them into naturally occurring and efficiently accumulated heme molecules by bacteria, where gallium ions are added to the active center instead of iron ions. Such gallium porphyrin derivative is efficiently taken up by bacterial cells via naturally occurring heme transport systems. The second functionality of gallium porphyrin derivatives is that they can be excited by visible light and generate reactive oxygen species (ROS), the so-called photodynamic effect.

For the past several years, our team has been working on the efficacy of gallium derivatives of porphyrins against human pathogens, mainly *S. aureus*, but also other representatives of the so-called ESKAPE group of pathogens. To characterize the activity of this group of antibacterial compounds, we tested various gallium porphyrin derivatives for production of ROS, accumulation in bacterial cells, and cyto- and phototoxicity against eukaryotic cells. We showed that they are transported by bacterial heme transport systems, so they can be precisely delivered to the cell. In this respect, they are therefore similar to antibiotics. On the other hand, their mechanism of action by generating non-specific ROS brings them closer to the action of disinfectants.

Photoantimicrobial activity of porphyrin-functionalised bioactive glass scaffolds

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Photoantimicrobial chemotherapy (PACT) is an antibiotic-free approach to inactivate microorganisms that relies on exposing the target microbes to a chosen photosensitiser and subsequently irradiating the infected area with light.[1,2] The resulting localised production of reactive oxygen species (ROS) damages subcellular structures leading to the death of the microorganisms. The high reactivity or ROS determines the non-specific destruction of any biomacromolecule near their site of generation: this gives PACT a broad spectrum of action (e.g., bacteria, fungi, viruses, protozoa, etc.) and a limited potential of inducing of drug resistance in the microorganisms.[3] The ability of ROS to inactivate microbes without penetrating the cell sparked interest in the association of photosensitisers with macromolecular support to obtain photoantimicrobial materials. Studies demonstrated the potential of these materials as antimicrobial surface coatings, wound dressing agents and materials for regenerative medicine.[4] In the latter field, in particular, the availability of a minimally invasive approach to eradicate bacterial infections of implants is particularly desirable, as it would remove the main cause of prolonged hospitalisation and implant failure.[5] With the aim of exploring the applicability of the photoantimicrobial approach to the eradication of bacterial infection on materials suitable for bone grafts,[6] we undertook the synthesis of bioactive glass scaffolds functionalised with porphyrin photosensitisers and studied the photoantimicrobial efficacy of the resulting conjugates against planktonic methicillin-resistant Staphylococcus aureus.

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Targeted bactericidal activity of ruthenium complexes by NO release under irradiation

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Cutaneous wound infection is a global issue as millions of patients get infected and millions of euros are spent on antimicrobial treatment each year, for preventive or curative purposes. The synthesis of a new family of antimicrobials has become crucial due to the current situation, in order to prevent persistent bacterial resistance from the new derivatives of already known and used antimicrobials and to offer new mechanism in the destruction of microorganisms. [1]

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Ruthenium nitrosyl (RuNO) complexes have shown efficiency as donors of nitric oxide (NO), known as a key mediator in biofilm dispersal. By reacting with oxygen, nitric oxide induces oxidative stress in microorganisms leading to their destruction, and its controlled release is responsible for antibacterial activity. [2]

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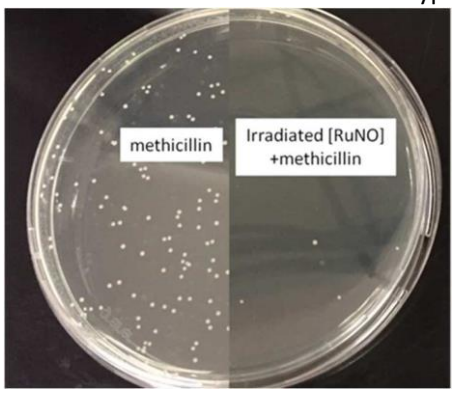
Herein, a light-controlled and targeted NO production through photo-activation of RuNO complexes was proposed as “non-traditional” antimicrobial agents with wound healing activity. [3] A library of Ruthenium-nitrosyl complexes with ligands of various photo-physical properties was synthesized and characterized. Antimicrobial spectrum was determined on several Gram (+) and Gram (-) bacterial strains. Moreover, cytotoxicity and phototoxicity effects were also determined on several types of skin cells.



Number of colonies of S. epidermidis ATCC 35984 after treatment with methicillin (left side) and with combined treatment of [RuNO]-methicillin (right side) in presence of irradiated [RuNO]. [RuNO] stands for trans (NO,OH)[Ru(FT)NO(OH)Cl] (PF_6). Irradiation was performed during 10 minutes with a Hg lamp (32mW) [2]

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Photodynamic inactivation with novel gallium metalloporphyrin – the non-antibiotic method to overcome *S. aureus* and *P. aeruginosa* biofilms

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Introduction: Approximately 60% of bacterial infections occur from a highly organized biofilm structure. Biofilm-forming bacteria exhibit much higher drug resistance than planktonic cultures. Biofilm bacteria express an altered set of genes and thus produce a variable microbial protein compared to bacteria in planktonic culture [1]. In the light-independent action, gallium metalloporphyrin has been reported to reduce biofilm formation and disrupt already-formed biofilms due to the presence of gallium ions [2]. In this study, the antimicrobial photodynamic inactivation (aPDI) with novel water-soluble gallium metalloporphyrin (Ga-CHP 2-3) was used as a method to treat both planktonic and biofilm culture of *Staphylococcus aureus* and *Pseudomonas aeruginosa* [3].

Materials and Methods: A water-soluble photosensitizer known as Ga-CHP 2-3 was used, which consists of Ga-PPIX (gallium protoporphyrin IX) with additional quaternary ammonium groups that increase its water solubility. A combination of Ga-CHP 2-3 and blue light (λ_{max} = 409 nm) was used to eliminate *S. aureus* Newman and *P. aeruginosa* ATCC 27853, both in planktonic form and in biofilm. The photosensitizer was incubated with the bacteria for 30 minutes in the dark, washed, and then irradiated with an appropriate dose of light (0-18.7 J/cm^2). Bacterial samples were serially diluted and cultured on agar plates for counting colony-forming units (CFU/ml).

Results: Bacterial eradication (reduction in bacterial viability >5log$_{10}$ CFU/mL) was observed for *S. aureus* (1 µM, 1.6 J/cm^2) and *P. aeruginosa* (5 µM, 6.2 J/cm^2) after aPDI treatment in planktonic culture. In contrast, in 24-hour biofilms, bacterial reduction was estimated to be 2 log$_{10}$ CFU/mL for *S. aureus* (5 µM, 18.7 J/cm^2) and 4 log$_{10}$ for *P. aeruginosa* (10 µM, 12.5 J/cm^2).

Conclusions: aPDI with novel gallium metalloporphyrin Ga-CHP 2-3 was an effective method to eliminate *S. aureus* and *P. aeruginosa* in planktonic cultures. In the case of biofilm, we observed a reduction corresponding to a 99% to 99.99% decrease in bacterial counts .

Acknowledgment: The work is supported by NCN grant no. 2018/30/Q/NZ7/00281(MG).

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Novel gallium photosensitizers in light-dependent action as an antivirulence strategy against the *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

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The Gram-positive microorganism *Staphylococcus aureus* and the Gram-negative *Pseudomonas aeruginosa* are associated with nosocomial infections, which are difficult to combat due to the arsenal of virulence factors that these microorganisms are disposing of (1, 2). Therefore, with the current study, we aimed at the involvement of two cationic photosensitizers (GaCHP 1-2 and GaCHP 2-3) and blue light (409 nm) against selected virulence factors of these clinical representatives: *S. aureus* isolate 5N, *P. aeruginosa* 133/k and reference strains. In addition, the *S. aureus* strains SA144, SA145, and SA147 were used to evaluate staphyloxanthin levels after exposure to tested conditions.

Exposure of all the bacterial strains to GaCHP 1-2 and GaCHP 2-3 in light-depended action (with the application of 409 nm) resulted in a significant decrease of bacterial viability (> 5 $\log_{10}$ CFU/ml) when both compounds were applied in concentration 10 µM. Furthermore, exposure of bacterial pigments to GaCHP 1-2, GaCHP 2-3, and 409 nm light decreased its levels in 133/k clinical isolate and SA147 and SA144 isolates. Furthermore, upon light-depended treatment with both gallium compounds, increased susceptibility to antibiotics to, e.g., doxycycline and chloramphenicol (in case of 5N isolate) and to ceftazidime, aztreonam (for 133/k clinical isolate) was observed. Lastly, the *P. aeruginosa* Toxin A activity upon its exposure to 10 µM GaCHP 1-2, and light dose 3.12 J/cm^2 was decreased. A similar observation was drawn for the same compound regarding staphylococcal enterotoxin C (SEC) production upon exposure to concentration 1µM and 6.24 J/cm^2.

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Melanin: the light and the dark

Invited speakers:

IL5 Tadeusz Sarna (Krakow,Poland)

The physicochemical basis for phototoxicity of melanin pigments

IL6 Michal Sarna (Krakow,Poland)

Photoprotective properties of melanin obtained from induced pluripotent stem cell-derived melanocytes

IL7 Sandra Del Bino-Nokin (Aulnay-sous-Bois, France)

Melanin content in skin of variable pigmentation and its impact on photoprotection

IL8 Andrea Hanel (Kuopio, Finland)

Skin colour and vitamin D: An archeogenomic approach

IL9 Antony R Young (London, UK)

The impact of melanin on vitamin D synthesis

Oral communications:

OC5 Alois W. Schmalwieser: Skin colour and pigmentation of Austrian farming families

OC6 Ana Borrego-Sánchez: Towards the understanding of the interaction of melanin dioxetanes with DNA

OC7 Joanna Turner: Estimation of erythemal radiation reflectance based on photopic weighted reflected irradiance

The physicochemical basis for phototoxicity of melanin pigments

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Although melanin is commonly viewed as a photoprotective pigment and efficient antioxidant, the ability of melanin to protect pigmented cells against phototoxicity maybe significantly modified by its oxidative degradation accompanying ageing. We have recently shown that photoreactivity and phototoxic potential of natural melanin from human hair substantially increased after partial photobleaching of the pigment granules, induced by aerobic photolysis [1]. To determine physicochemical basis of these changes, paramagnetic, optical, redox and photochemical properties of DOPA-melanin and cysteinyldopa-melanin – synthetic models of eumelanin and pheomelanin – subjected to experimental photodegradation, were analyzed by electron paramagnetic resonance (EPR) spectroscopy, EPR-spin trapping and EPR oximetry, UV-vis absorption spectroscopy, dynamic light scattering and atomic force microscopy, and time-resolved singlet oxygen phosphorescence. The measurements revealed that aerobic photodegradation of the synthetic melanins was accompanied by distinct changes of the physical properties of the melanin and chemical modifications of its key building blocks. These changes significantly increased the yield of the photodegraded melanin to photogenerate singlet oxygen and, to some extent, superoxide anion. The photodegraded melanins also exhibited substantially lower antioxidant capacity. The data suggest that photoaging of melanin, which presumably occurs in the human retinal pigment epithelium, could elevate the phototoxicity of melanin by increasing its ability to photogenerate reactive oxygen species and by reducing its antioxidant efficiency.

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Photoprotective properties of melanin obtained from human induced pluripotent stem cell-derived melanocytes

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Photoprotective properties of melanin are determined by its physicochemical parameters, which can be modified by a range of different factors, including cellular enzymes, exogenous chemicals, exposure to light, temperature, etc. Therefore, in order to evaluate the exact photoprotective potential of melanin, the pigment must be in its most native form. However, in most studies natural melanin was obtained from hair and pigmented tissues of the eye, enhancing the probability of substantial modification of the melanin by dehydration and photodegradation, which could affect the pigment physicochemical properties. To overcome these limitations, we employed an *in vitro* model based on the culture of human induced pluripotent stem cell-derived melanocytes (hiPSC-Mel) acting as a bioreactor for melanin synthesis under strictly controlled conditions. Using an array of spectroscopy and microscopy techniques, such as UV-Vis absorption spectroscopy, electron paramagnetic resonance, dynamic light scattering, atomic force microscopy and transmission electron microscopy we showed that melanin obtained from hiPSC-Mel had physicochemical properties is in its most native form. Moreover, DPPH assay and timeresolve singlet oxygen phosphorescence were used to measure antioxidant properties of the pigment. Finally, in a model cell system, employing human epidermal keratinocytes with phagocytized melanin we showed that the pigment has photoprotective properties greatly exceeding those of ocular and hair melanin. The results of our study indicate that melanin obtained from hiPSC-Mel is a good source of high quality pigment with exceptional photoprotective properties.

Melanin content in skin of variable pigmentation and its impact on photoprotection

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UV exposure is responsible for sunburn and DNA lesions that may be crucial for skin cancer development. Epidemiologic studies show higher incidence of basal and squamous cell carcinoma as well as melanoma in fair skin compared to dark. Skin color can be objectively classified in six groups - Very Light, Light, Intermediate, Tan, Brown and Dark - according to the individual Typology Angle (°ITA) based on colorimetric parameters of the CIE L*a*b* system. To understand the relationship between constitutive pigmentation and UV sensitivity, biological markers of UVB-induced erythema were analyzed and showed a significant correlation between ITA and sunburn cells as well as between ITA and DNA damage. DNA damage occurring specifically in melanocytes was also analyzed and showed greater damage in Light, Intermediate and Tan skin as compared to Brown and Dark skin.

To better understand the different UV response we characterized the melanin content in skin of variable pigmentation. Human skin contains two distinct components: brown to black, eumelanin and light colored, pheomelanin. Eumelanin consists of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) moieties, while pheomelanin consists of benzothiazine (BT) and benzothiazole (BZ) moieties. Eumelanin is photoprotective, by limiting the extent of UV penetration within the epidermis, and antioxidant, due to its reactive oxygen radicals scavenging capabilities. In contrast, pheomelanin has photosensitizing properties, leading to the UV-induced production of reactive oxygen species. Furthermore, within eumelanin, DHICA has superior antioxidant and free radical scavenger properties than DHI. Melanins can be quantitatively analyzed through specific degradation products by high-performance liquid chromatography (HPLC). Alkaline hydrogen peroxide oxidation (AHPO) of eumelanin gives rise to pyrrole-2,3,5-tricarboxylic acid (PTCA) and pyrrole-2,3-dicarboxylic acid (PDCA) as specific degradation products of DHICA and DHI moieties, respectively. Nevertheless, until recently, for eumelanin, only the DHICA content could be estimated. As a lately improved AHPO - HPLC method enabled a better characterization of PDCA, we reported the quantification of DHI eumelanin content in skin of varying pigmentation. Results confirmed respectively 76 and 24% eumelanin and pheomelanin content shown previously and revealed for the first time the ratio of 4 moieties: DHI 35%, DHICA 41%, BZ 20%, and BT 4%. The ratio is constant regardless of the degree of pigmentation. The high content of DHICA moiety in Brown and Dark skins may impart the melanin antioxidant property in the epidermis of these skin types thereby explaining their lower sensitivity towards UV rays.

Skin colour and vitamin D: An update

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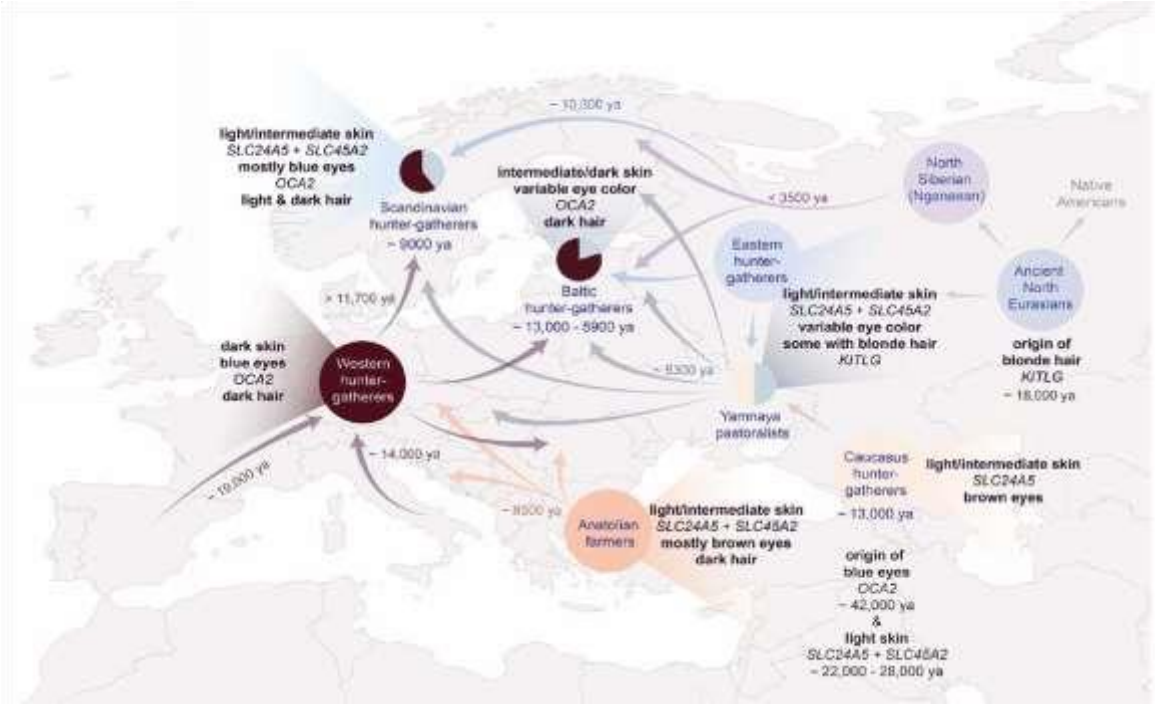
Homo sapiens evolved in East Africa and had dark skin, hair, and eyes, to protect against deleterious consequences of intensive UV radiation at equatorial latitudes. Intensive skin pigmentation was thought to bear the risk of inefficient vitamin D_3 synthesis in the skin. This initiated the hypothesis that within the past 75 000 years, in which humans migrated to higher latitudes in Asia and Europe, the need for vitamin D_3 synthesis served as an evolutionary driver for skin lightening. In this review, we summarize the recent archeogenomic reconstruction of population admixture in Europe and demonstrate that skin lightening happened as late as 5000 years ago through immigration of lighter pigmented populations from western Anatolia and the Russian steppe but not primarily *via* evolutionary pressure for vitamin D_3 synthesis. We show that variations in genes encoding for proteins being responsible for the transport, metabolism and signalling of vitamin D provide alternative mechanisms of adaptation to a life in northern latitudes without suffering from consequences of vitamin D deficiency. This includes hypotheses explaining differences in the vitamin D status and response index of European populations.



History of human pigmentation in Europe. Map shows migration and admixture of populations in Europe and their phenotype based on archeogenomics studies. Variations in the pigmentation intensity of skin, eyes and hair in Europeans are largely explained by SNPs related to the genes SLC24A5, SLC45A2, OCA2 and KITLG encoding for key proteins in melanogenesis. Ya, years ago

The impact of melanin on vitamin D synthesis

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Solar UVB radiation is essential for vitamin D synthesis in human skin and is much more important for vitamin D status than dietary intake. Melanin is a natural sunscreen and is very effective at protecting against sunburn and solar UVR-induced DNA damage to epidermal cells, especially in the stem cell containing basal layer (1). It is widely believed that early humans lost skin melanin as they migrated from Africa to less sunny climates to maintain the ability to synthesize vitamin D. The impact of skin melanin on vitamin D synthesis is a matter of controversy and few studies have been designed to quantify the inhibitory effect of melanin on vitamin D production. We undertook such a study by comparing serum vitamin D status ($25(OH)D_3$) in different Fitzpatrick skin types (FST) after five serial whole-body exposures, with intervals of three to four days, to solar simulating radiation (SSR) or narrowband UVB radiation (311nm). Importantly, the UVR doses were the same for all skin types and were sub-erythemal for the lightest FST. Analyses were based on comparing the slopes of the linear dose response curves for the different FST. Comparisons of FST II (white – sun sensitive) with VI (black – sun tolerant) showed melanin inhibitory factors (MIF) for 1.3 and 1.4 for SSR and 311nm respectively (2). These values are very much lower than the melanin protection factor of ~60 (1) in basal layer DNA damage (thymine dimers). The reason for the very modest effect of melanin on vitamin D synthesis may be that the chromophore for vitamin D synthesis (7-dehydrocholesterol) is present at high concentrations above the basal layer that contains the greatest concentration of melanin. Interestingly, the MIFs that we observed are in the region of the differences in melanin status and white and black populations.

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Skin colour and pigmentation of Austrian farming families

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Background: Farmers are chronically exposed to solar (ultraviolet) radiation. However, little quantitative data on resulting skin colour and pigmentation in farmers is available, nor on differences to other parts of the population.

Material and Method: Cutaneous tri-stimulus measurements (L*a*b*) on different body sites for one year were made in Austrian farming families (farmers, their spouses, and children). Pigmentation was estimated by the "Individual Typology Angle" (ITA°). Buttock skin was an appropriate reference during the entire time frame. Following this, we defined the "degree-of-tan" (TAN°) as the difference in the ITA° between constitutive (buttock skin) and facultative pigmentation.

Results: Adult males – independent of occupation – had a darker red component, especially in skin colour of the forehead, than adult females and children. The highest values were observed in males only. Analysis shows that this difference develops during puberty and adolescence. An obvious TAN° was found in all groups in late winter at continuously and intermittently exposed body sites. The TAN° was higher in adults than in children and highest in farmers. The TAN° shows pronounced seasonal changes in all groups on intermittently exposed body sites but less so on the forehead.

Conclusion: The TAN° increases in farmers during their lifetime. The highest TAN° values were found in farmers older than 50 years. The TAN° does not increase in their spouses, even though many spouses have higher TAN° than farmers of the same age.

Towards the understanding of the interaction of melanin dioxetanes with DNA

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Melanin exposed to oxidative conditions has been shown to produce dioxetanes that can induce DNA damage by chemical excitation and triplet sensitization. Under such conditions, peroxynitrite is produced and interacts with melanin leading to decomposition products such as 5,6-dihydroxyindole-2-carboxylic acid (DHICA). Subsequent reactivity between DHICA and peroxynitrite in the cell nucleus produces dioxetane derivatives, which decompose generating triplet excited states. This is followed by energy transfer from triplets to nucleobases and finally pyrimidine dimerization in DNA[1]. An important mechanistic aspect to understand this phenomenon is the binding/unbinding interaction of the DHICA dioxetane with the DNA. The intercalation of the molecule at the base pairs is expected to be particularly interesting because it could enhance the triplet energy transfer process. In this work, enhanced sampling techniques using GAMBES[2] and OPES flooding[3,4] methods have been used to compute the residence time of the DHICA dioxetane in the DNA and the binding/unbinding properties. The results obtained show that the release occurs on time scales of the order of μs. The simulations have allowed identifying different mechanisms of unbinding.

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Estimation of erythemal radiation reflectance based on photopic weighted reflected irradiance

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The beneficial and damaging impacts of ultraviolet radiation on the biosphere and biological systems have long been well-known. In the past, there has been limited research on the reflected ultraviolet (UV) irradiance from both natural and built surfaces. Even though reflectance from natural surfaces is relatively better understood, it is still ultimately of limited scope. A recent review (1) has summarized, for the first time, all known measurements of UV albedo and UV reflectance and demonstrated their influence on human UV exposure. The review recommended conducting further research to understand the nature of UV reflection from surfaces and learn how it impacts on UV exposure.

There is some evidence for the possibility of estimating the erythemal weighted UV reflectance for a specific material based on its photopic weighted reflectance. Previous work has shown only limited relationships between unweighted UV and photopic irradiance reflectance measurements (2), however new research suggests that there may be a correlation between reflected erythemal and photopic weighted irradiances. The new findings will be discussed and reviewed, and their significance for future research will be explored.

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Plants, UV radiation, and environmental change

Invited speakers:

IL10 Germar Bernhard (San Diego, USA)

The benefits of the Montreal Protocol for stratospheric ozone, UV radiation, climate, and the biosphere

IL11 Barry Lomax (Loughborough, UK)

Stratospheric ozone collapse, UV-B radiation, and the end-Permian mass extinction

IL12 Jorunn Olsen (Ås, Norway)

Does UV-B radiation affect plant responses to ionising radiation and vice versa?

IL13 Anthony Andrady (Rayleigh, USA)

UV Radiation, Microplastics, and Biology

Flash presentation (see poster sessions for abstracts):

Sheona Noemi Innes: Photochemical and growth response to inclusion of algal biomass in the growth medium of lettuce (Lactuca sativa).

The benefits of the Montreal Protocol for stratospheric ozone, UV radiation, climate, and the biosphere

Germar H. Bernhard[1]

[1] *Biospherical Instruments, Inc., San Diego, USA.*

The Montreal Protocol is an international treaty designed to phase out the production of ozonedepleting substances (ODS). The main goal of the treaty is to protect the Earth's stratospheric ozone layer, which shields life on our planet from harmful ultraviolet radiation (UV). In addition, the Montreal Protocol has mitigated climate change because ODSs, such as chlorofluorocarbons, are also potent greenhouse gases. The Montreal Protocol is therefore hailed as the most successful treaty to protect the biosphere both from increases in UV radiation and global warming. This presentation is based on the latest assessment prepared by the Environmental Effects Assessment Panel (EEAP) of the Montreal Protocol under the umbrella of the United Nations Environment Programme (Bernhard et al., 2023) and additional findings that were published since the assessment's cut-off date of September 2022.

Changes in UV radiation at low- and mid-latitudes (0–60°) during the last 25 years have generally been smaller than 4% per decade and were mostly driven by changes in cloud cover and atmospheric aerosol content. Without the Montreal Protocol, erythemal (sunburning) UV irradiance would have increased between 1996 and 2020 by 10–20% at mid-latitudes, by about 25% at the southern tip of South America, and by more than 100% at the South Pole in spring (Figure 1). Under the presumption that all countries will adhere to the Montreal Protocol in the future, erythemal irradiance at mid-latitudes is projected to decrease between 2015 and 2090 by 2–5% in the north and by 4–6% in the south due to recovering ozone. Furthermore, the phase-out of ODSs may have avoided warming by 0.5 to 1.0 °C over midlatitude regions of the continents, and by more than 1.0 °C in the Arctic. Stratospheric ozone depletion over Antarctica also led to a poleward shift of climate zones. Resulting changes in precipitation have affected ecosystems in South America and Australia.

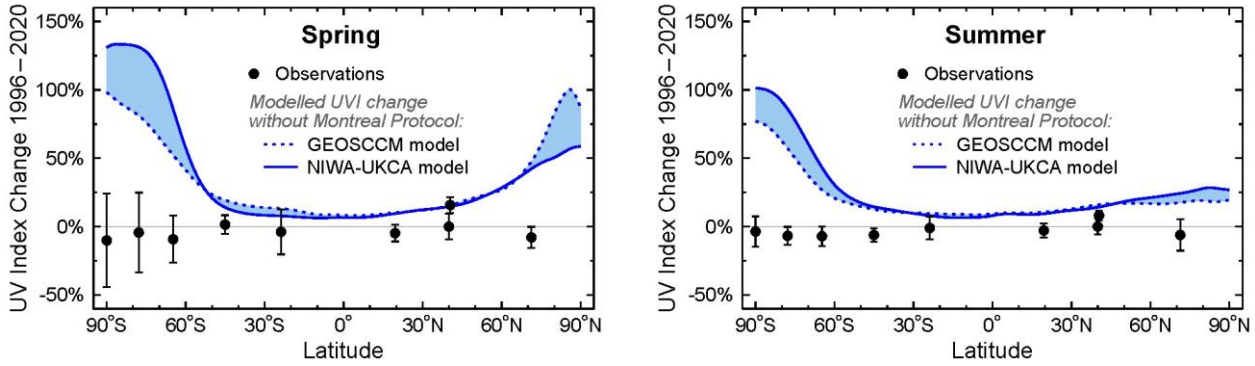

Figure 1: Comparison of observed changes in the UV Index between 1996 and 2020 (black circles) and changes without the Montreal Protocol estimated by two models (blue lines) for spring (left) and summer (right).

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Stratospheric ozone collapse, UV-B radiation, and the end-Permian mass extinction

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The sporomorph (pollen and spore) fossil record is one of the most complete archives of past life available to palaeontologists. This high-quality record is a function of sporomorphs being produced in vast numbers and the stability of the biopolymer, sporopollenin, making up the outer wall of the grains, which makes them resistant to decay. These factors, in combination with their ease of dispersal, means that they can be recovered from a wide range of rock types spanning both marine and terrestrial environments making them an ideal fossil to work on. Traditionally, this work has been based on identifying biological affinities of fossils with first occurrence being used to detect key innovations in the land plant phylogeny and their last occurrences have, for example, been used as a method to assess biodiversity loss in the face of carbon-cycle perturbations and concomitant climate breakdown resulting in mass extinctions. In essence, the sporomorph record has been used as a passive archive for monitoring species occurrence, abundance and diversity over time. But, more recent work has highlighted that the sporomorph fossil record can be used in a more dynamic way to tease out environmental stresses. In this talk I will outline how, by linking the biogeochemical analysis of sporomorphs with their morphology, specifically high abundances of malformed grains have enabled us to develop a more holistic understanding of the end-Permian extinction event. The talk will focus on demonstrating how the collapse of the stratospheric ozone layer and the concomitant increase in the flux of deleterious UV-B radiation may have played an important role in this iconic event, the most significant [to date] mass extinction event of the last 540 million years.

Does UV-B radiation affect plant responses to ionising radiation and vice versa?

Jorunn E. Olsen[1,2], Dajana Blagojevic[1,2], YeonKyeong Lee[1,2], Li Xie[1,3], Dag A. Brede[2,4], Line Nybakken[2, 4], Ole Christian Lind[2,4], Knut Erik Tollefsen[1,3], Brit Salbu[2,4], Knut Asbjørn Solhaug[2,4]

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UV radiation plays important roles in plant development and is known to increase tolerance to various stressors. UV and ionising radiation have differences but also commonalities in that both induce formation of reactive oxygen species (ROS) and DNA damage. However, information about interactive effects on plants is sparse and we aimed to explore this. Exposure of young Scots pine (*Pinus sylvestris*) seedlings to UV-B (0.35 or 0.5 W m^{-2}; 10 h daily) and gamma radiation (10-125 mGy h^{-1}) for 6 days, with or without UV-B pre-treatment for 4 days, resulted in a gamma-dose rate dependent increase in DNA damage from 10 mGy h^{-1}, generally with additional UV-B-induced damage (Blagojevic et al. 2019). However, in contrast to UV-B and regardless of its presence, gamma irradiation at ≥ 40 mGy h^{-1} resulted in decreased shoot growth and increased ROS level. There was no effect on total antioxidant capacity although UV-B induced some phenolic compounds. Thus, there was no evidence of a protective effect of UV-B on gamma-induced growth-inhibition and DNA damage in Scots pine and no additive adverse effect on growth. In comparison, UV-B (0.5 W $^{-2}$) and gamma (15-40 mGy h^{-1}) irradiation of *Lemna minor* for 7 days did not result in any interactive, additive effect on growth and antioxidant gene expression but synergistic effects were observed for reproductive inhibition and some photosynthesis-related parameters, and antagonistic effects for some oxidative stress parameters (Xie et al. 2022). These results indicate that interactive effects of UV and ionising radiation may vary between biological processes and species and may be influenced by exposure conditions. This underlines the need for further studies.

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UV Radiation, Microplastics, and Biology

Anthony Andrady

Department of Material and Biomolecular Engineering North Carlina State University, Raleigh, NC. USA

The talk will relate the role of solar UV radiation in generating microplastics and nanoplastics in the environment. Microplastics are now a ubiquitous environmental contaminant, especially in oceans and rivers worldwide. Ingestion of these micro-fragments by marine animals as well as human consumers is a serious concern, especially because of their physiological impacts, likely to be species-dependent, are as yet unclear. Microscale plastics in human blood and placenta, for instance, have recently been reported.

The role of solar radiation in generating secondary microplastics from plastic litter is well established. Common plastics are susceptible to this process, and the mechanism of their degradation and fragmentation will be presented. Some of the critical gaps in knowledge on the interaction of common plastics and UV radiation that need to be urgently addressed will also be discussed.

Bioluminescence

Invited speakers:

IL14 Elisa Michelini (Bologna, Italy)

The contribution of bioluminescence to the 2030 Agenda for Sustainable Development: novel biosensors for point-of-need applications

IL15 Daniel Roca-Sanjuan (Valencia, Spain)

On the electronic-structure features of the chemical excitation phenomena

IL16 Isabelle Navizet (Paris, France)

Bioluminescent systems: challenges in modelling their absorption and emission spectra

IL17 Martin Marek (Brno, Czech Republic)

Illuminating the mechanism and allosteric behavior of NanoLuc luciferase

Oral communications:

OC8 Sergio Adan-Bermudez: Detection of endogenous porphyrins in bacteria using hollow-core photonic crystal fibres

OC9 Houda Moumene: QM/MM calculations and spectra modelling of the Nanoluc-Furimamide bioluminescent system

OC10 Juliana Cuéllar-Zuquin: Unravelling the mechanism of melanin dioxetane chemiexcitation in the context of DNA damage

OC11 Josep Alberola Boloix: Chemiexcitation of indole-like biomolecules and their role in DNA damage: a quantum-chemistry perspective

Flash presentations (see poster sessions for abstracts):

Mathilde Seinfeld: A joint experimental and computational study on the influence of packing on organic compounds luminescence and singlet oxygen generation in the condensed phase

Dario Santantonio: Developing a SWIR/NIR Imaging System for Singlet Oxygen Detection in Biologically Relevant Environments

The contribution of bioluminescence to the 2030 Agenda for Sustainable Development: novel biosensors for point-of-need applications

Elisa Michelini [1,2], Denise Gregucci [1,2], Maria Maddalena Calabretta[1,2] [1]

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We are currently facing enormous challenges connected to the growing and aging population, the protection of the environment and the climate together with the need to ensure food safety and quality.

The 2030 Agenda for Sustainable Development, adopted by all United Nations Member States in 2015, identified 17 sustainable development goals (SDG)as a universal call to action to face such challenges. In this context bioluminescence, i.e., the emission of light in living organisms, can make an important contribution. Thanks to advancements in synthetic biology, organic chemistry and computational models, today bioluminescence has become a powerful tool for a plethora of applications including biosensors, bioassays and in vivo imaging. These bioanalytical tools can be vital to tackle several SDG, such as SDG2 (zero hunger), SDG3 (good health and well-being). SDG6 (clean water and sanitation) and others.

Here we report the generation of a portfolio of biosensors based on living cells (bacteria, yeast, and human cell lines grown in 3D cultures)[1] and cell-free systems that were integrated into smartphonebased devices and paper sensing platforms for rapid on-field detection of analytes of environmental, food, forensic and clinical interest. Whole-cell biosensors relying on multiplexed reporter gene technology and split complementation strategies were integrated into 3D-printed cartridges and smartphone-based biosensors were developed and applied to the detection of different bioactivities in water samples. These biosensors have been also "upgraded" with the implementation of new luciferase mutants characterized by improved thermostability at 37°C, pH-insensitive emission, extended kinetics and red-shifted emission.

As an alternative, cell-free systems were also developed, by exploiting either purified luciferases or the NanoLuc Binary Technology to detect microbial contamination, pesticides and other pollutants in water, food and other complex biological matrices. The combination of luciferases with nanomaterials, i.e., metal-organic framework (MOF)[2], to improve the stability and robustness of the analytical platforms has been also explored and proof-of-principle applications of these biosensors are presented together with main limitations and current challenges to turn them into marketable biosensors.

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On the electronic-structure features of the chemical excitation phenomena

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Light absorption promotes most of the phenomena presented in this congress on photobiology. Excited electronic states are generated upon light irradiation, which adds extra energy into the molecular systems and allows highly-energetic chemical reactions. Such excited states can be also generated by a special type of thermal reactions, normally based on a cyclic peroxide decomposition. This phenomenon is named chemical excitation or chemiexcitation. It is happening for instance in bioluminescence, in which the excited state decay by light emission, and has plenty biological functions in the living organisms. Moreover, it is involved in the DNA damage production in dark conditions.

In the last decade, our research group (Quantum Chemistry of the Excited States – Universitat de València, QCEXVAL) has been applying quantum chemistry and molecular modeling to figure out which are the key electronic structure characteristics of the chemiexcitation process in the context of chemiluminescence, bioluminescence and photochemistry in the dark [1,2]. In this talk we shall see the main conclusions that we got so far.

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Bioluminescent systems: challenges in modelling their absorption and emission spectra

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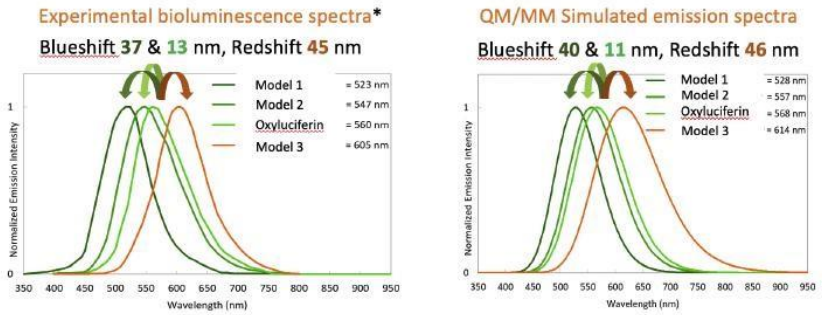
The emitting light in fireflies or other bioluminescent species arises from the electronic relaxation of oxyluciferin, an organic compound resulting from the oxidation of the D-luciferin substrate inside an enzyme called luciferase. These systems are already used in applications such as cell cancer detection.

One of the challenges of the modelling of such systems, like other biological photoreceptor, is to reproduce the experimental absorption, fluorescence and emission spectra in order to better interpret the experimental results.

Here will be presented the methodologies that can be used to study the spectroscopic proprieties of bioluminescent systems using quantum mechanics, molecular dynamics and hybrid (QM/MM) methods. The experimental emission and absorption spectra are accurately reproduced when the dynamic of the system is taking into account. Information of the nature of the transition can be taken from the modelling and insight of the influence of the protein environment can be highlighted.



Comparison of experimental and theoretical spectra for oxyluciferin and analogues

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Illuminating the mechanism and allosteric behavior of NanoLuc luciferase

Michal Nemergut[1,2], Daniel Pluskal[1], Jana Horackova[1], Tereza Sustrova[1], Jan Tulis[1], Tomas Barta[3], Veronika Novakova[1,2], Marika Majerova[1], Karolina Sedlackova[1,2], Sérgio M. Marques[1,2], Martin Toul[1,2], Jiri Damborsky[1,2], David Bednar[1,2], Zbynek Prokop[1,2], Yves L. Janin[4], and Martin Marek[1,2,*]

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4. *Structure et Instabilité des Génomes (StrInG), Muséum National d'Histoire Naturelle, INSERM, CNRS, Alliance Sorbonne Université, 75005 Paris, France*

* Speaker and corresponding author: Martin Marek (martin.marek@recetox.muni.cz)

NanoLuc, a superior β-barrel fold luciferase, was engineered 10 years ago but the mechanism of its action remains puzzling. In this report, we combined experimental and computational techniques, revealing that imidazopyrazinone luciferins bind to an intrabarrel catalytic site but also to an allosteric site shaped on the enzyme surface [1]. Binding to the allosteric site prevents simultaneous binding to the catalytic site, and vice versa, through concerted conformational changes. We demonstrate that restructuration of the allosteric site can boost the luminescent reaction in the remote active site. Mechanistically, an intrabarrel arginine coordinates the imidazopyrazinone component of luciferin to attack O_2 via a radical charge-transfer mechanism, while it protonates the excited amide product to secure high emission intensity. Concomitantly, an aspartate, supported by two tyrosines, anchor and fine-tune the luciferin molecule inside the catalytic pocket, positioning it for productive catalysis. Thus, we show that NanoLuc, despite its structural dissimilarity, employs analogous tricks to secure a blue light-emitting phenolate anion, as we recently revealed for *Renilla*type luciferase [2]. This information is critical to engineering the next-wave of luciferaseluciferin pairs for ultrasensitive bioassays.

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This work was supported by the Czech Science Foundation (22-09853S).

Detection of endogenous porphyrins in bacteria using hollow-core photonic crystal fibres

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The overuse and misuse of antibiotics has led to the emergence and spread of antibiotic-resistant bacteria, which are a major cause of morbidity and mortality globally. Blue light photoinactivation is one of the most promising solutions, activating endogenous photosensitisers and leading to the generation of reactive oxygen species that can cause cell damage and death. The most effective area of radiation for photoinactivation in the visible is around 405-410 nm, a region that corresponds to the absorption maximum of the Soret band in porphyrins.

Quantifying the concentration of endogenous porphyrins in bacterial samples is necessary to assess their potential as diagnostic biomarkers and for photodynamic therapy. Several techniques have been used including fluorescence microscopy, high-performance liquid chromatography (HPLC), and mass spectrometry (MS). However, the very low concentration of porphyrin (0.1 – 1 nmol/mg), high doses of light irradiation, and the scattering of bacteria make this detection very challenging.

Hollow-core photonic crystal fibres (HC-PCFs) are state-of-the-art optofluidic systems that provide ultrasensitive detection. In HC-PCFs, light and liquid can be guided simultaneously through the fibre core. Light is guided with very few losses over long distances (<1 dB/km); and the total volume of the fibre, in the order of nanolitres, allow the detection of metabolites in the nanomolar range. The potential of HCPCF for applications in photochemistry has been already tested with the detection of fluorescence [1] with attomole sensitivity and sensing luminescence of singlet oxygen at 1270 nm in aqueous solution [2].

We report the use of HC-PCFs for the detection of endogenous porphyrins within bacteria solutions in the fibre core. This novel approach exploits the long path-length over in which signals of porphyrins can be trapped and guided to the detector. Overall, using PCFs, the signal detected is increased over six orders of magnitude, compared to conventional detection methods (e.g. cuvette). And this gives us the edge to quantify the concentration of porphyrins in bacteria.

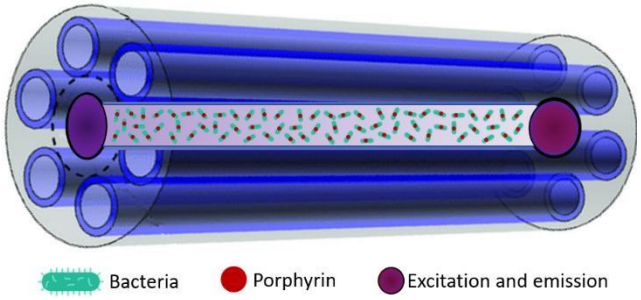

Figure 1. Cross-section of a PCF as an optofluidic sensor of endogenous porphyrins in bacteria.

Acknowledgment: This work was supported by the European Union's Horizon 2020 research and innovation programme under grant agreement nº 863102. www.light4lungs.eu

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QM/MM calculations and spectra modelling of the Nanoluc-Furimamide bioluminescent system

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Bioluminescence is a biological phenomenon of light production by living species with many applications[1], notably for the detection of cancer cells[2]. The present study deals with a ligand-protein system (furimazine – Nanoluciferase) derived from a shrimp called *Oplophorus gracilirostri* with a very high luminescence intensity[3]. The protein catalyzes an oxidation reaction of furimazine by dioxygen, which leads to a new molecule, the furimamide in its excited state. A better understanding of this bioluminescent system will provide insights to tailor new devices with emission of a photon with a red color, and thus a light signal more easily detectable inside the human body.

Simulations of the emission and absorption spectra are relevant tools[4] to comply this goal. We focus here on the absorption spectra of the light emitter system furimamide – Nanoluciferase. In order to take into account the protein environment and the system flexibility, we carry out classical (at the molecular mechanic level MM) molecular dynamics (MD) with AMBER software. Furimamide is a flexible molecule: standard force fields such as GAFF[5] (General Amber Force Field) are unable to reproduce correctly torsion angles potential energy when compared to in vacuo DFT computations. Thus, we perform a multidimensional fit using 1D and 2D potential energy surface scans with several torsion angles of the molecule in order to parameterize a new force field. Then, we compute electronic transitions at a QM/MM level of theory on a set of 100 snapshots extracted from each MM trajectory. We present the obtained absorption spectra of the system with different force fields (GAFF2 and new force field). We analyze also the MD simulations and compare the influence of the initial conformation on the interaction inside the cavity and on the spectra. As a perspective, we plan to use the same methodology to simulate emission spectra.

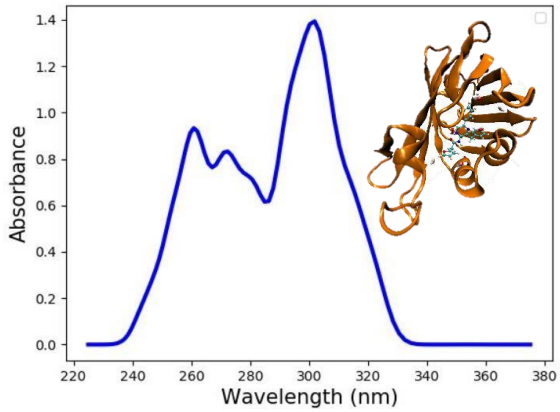

Figure. Theoretical absorption spectra of the Nanoluciferase –Furimamide. Representation of the system: furimamide (colored by atom type) inside the cavity of Nanoluciferase protein (in orange)

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Chemiexcitation of indole-like biomolecules and their role in DNA damage: a quantum-chemistry perspective

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Nucleobases in excited electronic states are involved in certain types of DNA damage. These molecules can be excited due to UV absorption, but also due to chemiexcitation reactions. One of the most relevant types of DNA damage formed in these conditions is the cyclobutane pyrimidine dimers (CPD). Recently, it was found that, under oxidative stress conditions, a melanin decomposition product, 5,6-dihydroxyindole-2-carboxylic acid (DHICA), was involved in the formation of CPD [1]. This was related to the formation of dioxetane species that decomposed in carbonylic compounds in a triplet excited state. Other biologically relevant molecules, such as tryptophan and serotonin might also cause CPD when exposed to reactive oxygen species [2,3]. Characterizing these dioxetane decomposition reactions is interesting, in particular, the step at which the excited molecules are formed. This would allow to discern whether a molecule could take part in DNA damage by chemiexcitation or not. In this work, the dioxetane decomposition energy profile of DHICA and other biomolecules with indole moieties was studied using quantum chemistry methods. One of the most relevant results is that tryptophan has an activation energy similar to DHICA. Although further studies must be done, this could indicate that oxidative stress can cause certain essential biomolecules to behave in ways that are detrimental to living organisms.

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Unravelling the mechanism of melanin dioxetane chemiexcitation in the context of DNA damage

Juliana Cuéllar-Zuquin[1], Rafael García-Meseguer[1], Josep Alberola-Boloix[1], Ana Borrego-Sánchez[1], Antonio Francés-Monerris[1], Daniel Roca-Sanjuán[1]

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Melanin, the pigment responsible for skin, hair, and eye coloration, has long been recognized for its photoprotective and antioxidant properties.[1] However, recent studies have shed light on an emerging "dark" role of melanin in the development of pyrimidine dimeric lesions in DNA through a process known as chemiexcitation.[2] Chemiexcitation is generally due to the decomposition of a dioxetane ring,[3] and in this case it occurs when a dioxetane derivate from melanin decomposes generating an excited molecule capable of transferring energy to nucleobases allowing their cycloaddition in absence of light. Interestingly, this "dark" pathway to DNA damage may have implications in the pathogenesis of various degenerative diseases, including Alzheimer's, Parkinson's, and age-related macular degeneration.[4]

In the present study, we employed computational chemistry tools to investigate the potential of 5,6dihydroxyindole-2-carboxylic acid (DHICA) in DNA chemiexcitation. DHICA is a melanin precursor proposed by Premi and co-workers in the formation of cyclobutane pyrimidine dimers (CPDs), which are well-known mutations responsible for the development of skin cancer lesions. Our research aimed at shedding light on the underlying mechanism of DNA chemiexcitation.

In particular, we conducted an investigation to determine which factors may affect the energetic barriers associated to the dioxetane ring decomposition and to explore whether catalysis of such reactions is possible within the biological environment. To achieve this, we modeled DHICA aggregates with various molecules possessing different ionization potentials, primarily nucleobases. We also varied the molecular orientations within the aggregates and studied the process in a hydrated double helix of DNA. The findings allow to evaluate the impact of π-π stacking interactions, hydrogen bond formation and spin delocalization in the chemiexcitation phenomenon.

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Parallel symposia

Monday August 28

Afternoon

Photoprotection

Symposium sponsored by **NAOS**

Invited speakers:

IL18 Hamid-Reza Rezvani (Bordeaux, France)

DNA repair-deficient models, useful tools for assessing the mechanism of natural photoprotection and property of photoprotective agents

IL19 Sandra Trompezinski (Aix-en-Provence, France)

In vivo biomarkers study to evaluate photoprotection products

IL20 Antony R. Young (London, UK)

Light sources for phototesting of sunscreens

IL21 Yolanda Gilaberte (Zaragoza, Spain)

New developments in UV filters

IL22 Henry Lim (Detroit, USA)

Personalized photoprotection

IL23 Salvador González Rodríguez (Madrid, Spain)

Natural photoprotective agents

Oral communications:

OC12 Furkan Akif Ince: Photoprotection by Vitamin D Compounds: Identifying Markers that Predict Efficacy in Preventing Carcinogenesis

OC12 Carine Jacques: A multiomic approach to understand solar exposure impact on skin ecosystem and evaluate a new SPF50+ sunscreen efficacy

DNA repair-deficient models, useful tools for assessing the mechanisms of natural photoprotection and property of photoprotective agents

Joudi El Mir[1] , Sandrine Fedou [1] , Nadine Thézé[1] , Julie Scalia[3] , Eric Perrier[3] , Walid Mahfouf[1] , Elodie Muzotte[1] , Jérôme Rambert[1,2], Sandra Trompezinski[3] , Pierre Thiébaud[1] , Muriel Cario[1,2] , Hamid-Reza Rezvani[1,2*]

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Ultraviolet B (UVB) radiations in sunlight cause skin damage, ranging from erythema, wrinkles to photoaging and skin cancer. To overcome the deleterious effects of UVB radiation, various photoprotective mechanisms, including constitutive and UV-induced pigmentation, stratum corneum thickness, UV-induced immune responses, UV-induced apoptosis, antioxidant defense, and DNA repair systems, have evolved in living organisms. One of the main features of UVB is affecting genomic DNA by creating cyclobutane pyrimidine dimers (CPDs) and pyrimidine–pyrimidine (6–4) photoproducts (6–4PPs). These lesions are mainly repaired by the nucleotide excision repair (NER) system and by photolyase enzymes. The importance of correctly removing UVB-induced lesions is demonstrated in patients with deficiency in the NER system, such as xeroderma pigmentosum (XP), who present pigmentary abnormalities, skin photosensitivity, accelerated photoaging and a predisposition to early onset skin cancers. To investigate the cellular responses to UVB, several models have been used including monolayer cells, skin equivalent, skin explant, and mouse models. Several aquatic species, ranging from crustacean and echinoderm to different species of fish and amphibian, have been also used to investigate the effects of UV radiation on development and behavior. Of note, increasing UVB radiation due to ozone layer depletion and consequently persistent UVB-induced damage have been proposed as a potential cause for the disappearance of amphibian populations. However, very few studies have addressed the comprehensive biological responses to UVB radiation, with respect to the natural photoprotection systems including DNA repair systems, skin responses, and melanocyte behavior. So, we wondered whether Xenopus laevis could be an appropriate in vivo model system for investigating the impact of UVB on skin. Overall, a gradual decrease in CPD levels, detection of apoptotic cells, thickening of epidermis, and increased dendricity of melanocytes emulate human skin responses to UVB and support Xenopus as an appropriate and alternative model for such studies. To go further, we then generated the DNA repair-deficient Xenopus embryos using morpholinos (antisense oligonucleotides that can specifically hybridize with a specific mRNA) against XPC mRNA. Our results indicated that the pigmentary pattern of XPC morphants were different from the control embryos. In parallel, we generated XPC-deficient primary human melanocytes using CRISPR-Cas9 technology, in order to investigate the photoprotection systems in DNA-repair deficient melanocytes and to avoid animal models use. Following validation of model (absence of XPC expression, DNA repair deficiency, and sensitivity to UV), XPC-deficient primary human melanocytes and their control counterparts were subjected to proteomic analysis. Results revealed that 496 proteins are differentially expressed in these cells. A protein-protein interaction (PPI) network was then constructed using IPA and suggested that several differentially expressed proteins (DEPs) contribute to 'cytoskeletal rearrangement', 'formation of vesicles and melanosomes', 'trafficking of vesicles and melanosomes', and 'melanin synthesis', which all could be related to pigmentary abnormalities in XPC-deficient cells. In conclusion, DNA-repair deficient models are valuable tools for studying the cross-talk between different photoprotection systems and to evaluate the efficacy of photoprotective agents.

In vivo biomarkers study to evaluate photoprotection products

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[1]NAOS ILS, Aix-en-Provence, France ; [2]NAOS, Aix-en-Provence, France ; [3]NAOS, Lyon, France ; [4]QIMA Synelvia, Labège, France

Chronic exposure to ultraviolet (UV) irradiation causes immunosuppression, photoaging, and carcinogenesis. A cascade of reactions occurs upon UV exposure of human skin, including the generation of reactive oxygen species (ROS), oxidation of lipids and proteins, DNA damages, p53 mutations, sunburn cell formation, release of cytokines and matrix metalloproteinases (MMPs). UV rays have effect on the cutaneous immune system by causing its suppression which can make the organism more vulnerable to bacterial, viral or fungal infections and to the development of cutaneous cancers. This action is linked to the migration of Langerhans cells from the epidermis, key cells of the cutaneous immune response and a reduction in the antigen presentation function. In addition, under UVB exposure, urocanic acid, a natural filter present in the stratum corneum, is trans-isomerized to form UCA-cis which also contributes to local immunosuppression. The skin barrier function is also affected by solar radiation. The stratum corneum (SC), which is the first line of defense against environmental exposures, is modified by solar radiation, with alterations in its mechanical properties and related barrier function mechanisms including filaggrin proteolysis. UV rays also affect the skin microbiome, whose protective role is essential to prevent photoimmunosuppression and good skin health.

Skin photoprotection has become a real public health issue. As a complex ecosystem, it is necessary to protect the skin from the effects of UVA, in addition to UVB. For many decades the SPF has assessed *in vivo* sun protection using UV-induced erythema as an endpoint, but it does not accurately reflect all photoprotection benefits.

In our research activities, we have sought to go further in evaluating the efficacy of sunscreen products by going beyond the SPF, thanks to *in vivo* quantification of skin biomarkers which reflect activation of cutaneous biological pathways and can be analyzed from non-invasive skin samples.

The aim of our *in vivo* study was finally to evaluate the photoprotective complementary efficacy of an active complex with sun filters on volunteers exposed to UVB and UVA by measuring squalene oxidation, trans-urocanic acid (trans-UCA) and a pool of amino-acids by LC-MS and catalase activity by quantification of resorufin fluorescence. Moreover, the impact of UV and the protective effect of sun filters coupled with active complex on human and bacterial proteins was analyzed using "shotgun proteomics" (LC-MS/MS).

In this context, we confirmed the interest of providing specific ingredients whose activity is complementary to that of UV filters to have a global full photo-protection against the negative effects of UV. A better understanding of *in vivo* cutaneous effects of solar irradiation is essential to optimally assess the efficacy of products and thus develop suitable sun photo-protection products.

Light sources for phototesting of sunscreens

Antony R Young

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Sunscreens are designed to attenuate solar ultraviolet radiation (UVR) and to some extent visible radiation (VR). Their efficacy is primarily tested on human volunteers using artificial sources of UVR under laboratory conditions. For practical reasons the irradiance of these sources is much greater than solar UVR. Their spectral properties are well defined by regulatory bodies and classified as either (i) solar simulated radiation (SSR) to determine sun protection factor (SPF) that is a measure of protection against erythema or (ii) UVA to determine UVA protection factor (UVA-PF) that is a measure of protection against persistent pigment darkening (PPD), the biological significance of which is unknown. The action spectrum for erythema is heavily UVB dependent, and therefore SPF is primarily, but not exclusively, a measure of UVB protection. UVA protection, relative to UVB, can also be assessed spectrophotometrically by measuring the optical properties of a sunscreen. The basic principle for human testing is to determine the UVR dose for a given endpoint with and without sunscreen application, at a given thickness, and the ratio of these doses is the protection factor. SSR spectra are UVB rich compared to solar UVR. This UVB content, even when within tolerances set by regulatory authorities, may impact SPF especially if the sunscreen has relatively poor UVA protection. Laboratory determined SPF may be used for ranking products by the consumer, but its relationship with protection against erythema from sunlight solar is uncertain. "Real life" SPF depends on many factors including sunscreen application thickness, but emission spectrum is likely to play an important role. There is increasing concern that laboratory SPF overestimates protection from natural sunlight (1), which is logistically difficult to determine. The reasons for this are poorly understood, but one possible explanation is that SSR and solar UVR spectra are different, with the former lacking in very long wave UVA and VR that may contribute to erythema (2). We clearly need a better understanding of the relationship between SPF with solar UVR and SSR.

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New developments in UV filters

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Over the years, significant advancements have been made in the development of UV filters to enhance their efficacy, especially in the long UVA range. Effective photoprotection is necessary because our skin is exposed 20 times more to UVAR than to UVBR. In addition, different significant biological and clinical effects are caused by this radiation such as damage to the DNA, photoimmunosupression, oxidative stress and hyperpigmentation. Some efforts have been made in the last years to develop new filters that enhance the photoprotection in the long UVA and blue light range. Two new filters are already in use, methoxypropylamino cyclohexenylidene ethoxyethylcyanoacetate (meroxyl 400) which provides a very good photoprotection in the UVA1 range, and phenylene bis-diphenyltriazine (triasorb) that provides protection also in the UVA1 and the blue light. Also under research, bis-(diethylaminohydroxybenzoyl benzoyl) piperazine (BDBP) that absorbs between 350 and 425 nm has also demonstrated to effectively protect against hyperpigmentation.

Furthermore, efforts have been made to improve the stability and longevity of UV filters under various conditions. Encapsulation technologies, such as microencapsulation and nanoencapsulation, have been utilized to protect UV filters from degradation caused by factors like light, heat, and interaction with other cosmetic ingredients. This approach ensures the sustained release of UV filters, extending their photoprotective performance over prolonged periods.

In conclusion, ongoing research and development in the field of UV filters have resulted in significant advancements in terms of enhanced photoprotection and sustainability.

Personalized photoprotection.

Henry W. Lim.

Department of Dermatology, Henry Ford Health, Detroit, Michigan, USA

It is well recognized that UVB (290-320 nm) induces sunburn response on the skin, with UVA2 (320-340 nm) also contributing to this biologic effect. UVA (320-400 nm) is the predominant spectrum that induces tanning response and photoaging process. Both UVB and UVA are responsible for photocarcinogenesis. In the past few years, significant understanding of the effects of visible light (VL, 400-700 nm) has been achieved. VL induces intense and persistent pigmentation, and UVA1 (340-400 nm) synergistically contributes to this effect. The pigmentary effect of VL + UVA1 occurs only in dark skinned subjects. It should be noted that exposure to VL + UVA1 results in transient erythema in both dark and light skinned subjects (ref 1) .

With the exception of several newly developed and introduced filters, the vast majority of filters used in sunscreens are not designed to protect against the effect of VL. Currently, protection against VL can be achieved in: i. Tinted sunscreens, which incorporate iron oxides and in some, non-micronized titanium dioxide; ii. Sunscreens containing biologically active antioxidants; and iii. Sunscreens with newly developed filters (Mexoryl 400, TriAsorB, and BDBP – with the latter not yet introduced in commercially marketed products as of June 2023). (ref 2)

Comprehensive photoprotection strategy includes seeking shade with outdoors, wearing photoprotective clothing, wide brimmed hat and sunglasses, and applying sunscreens on otherwise exposed skin. With worldwide population consisting of different skin tones, the concept and practice of personalized photoprotection regarding sunscreen recommendations is essential (ref 3). Individuals of lighter skin types have higher risk of UV erythema and photocarcinogenesis, the use of high SPF (50+), broad spectrum sunscreens is important. For dark skinned population, while the risk of sunburn and photocarcinogenesis is lower, they are more prone to develop UV and VL induced hyperpigmentation; therefore, sunscreens with lower SPF (30) can be recommended, however, it is essential that sunscreens with excellent UVA and VL protection are used.

Personalized photoprotection would allow us to provide appropriate photoprotection advice to all individuals.

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Natural photoprotective agents

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Skin cancer poses a significant public health problem, and its incidence is increasing steadily worldwide. Traditional sunscreening agents are a critical component in all photoprotective regimens and constitute its first prevention line; however, they have limitations and have also increasingly been questioned for their safety (harming our own health) and their impact on the environment (including harming coral reefs). Besides conventional filtering agents, a group of photoprotective agents of natural origin (namely Actives), primarily botanical, with demonstrated antioxidant, free-radical scavenging ability has drawn considerable attention. Many of these botanical derivatives including tea extract, such as epigallocatechin gallate, flavonoids, Polypodium leucotomos extract, pycnogenol, and lycopene) have shown to contrast photocarcinogenesis, as well as sunburn and photoaging in UVexposed hairless mice. Few however have also shown skin benefits in humans, such as protection against oxidative stress, DNA photodamage, and Langerhans cells UV-induced depletion, all of these are relevant mechanisms for skin photocarcinogenesis. Additionally, they also impact skin photoaging and photoinduced pigmentation, the latter of which is very significant in individuals of dark skin phototypes. Additional natural photoprotective agents are those from marine sources (algae, coral, among others) including mycosporine like aminoacids (MAAs-Palythine).

It is interesting to note that these natural photoprotectants can not only be administrated topically, but also by orally. Orally administered botanic photoprotectors could help to overcome many limitations of topical photoprotection, since their efficiency is not altered by external conditions (rubbing, sweating), their half-lives can be determined pharmacologically, and their effects do not depend on the degree of absorption into the skin. Oral natural photoprotectants may complement conventional photoprotection bringing very interesting and even synergistic effects, resulting in a positive impact on skin.

In this presentation, a narrative state of the art review will be performed contextualizing the description of current scientific thinking about the photoprotective capacity of a selected series of agents from botanical and marine sources. Their topical and oral use has been explored as a means to deal with solar radiation-induced oxidative stress, UV and Visible light in particular, reducing the damage caused at least partially by reactive oxygen species (ROS), impeding or lessening tissue damage including hyperpigmentation, and promoting DNA repair among other beneficial effects. The most promising natural photoprotective agents with scientific evidence in humans will be discussed.

Photoprotection by Vitamin D Compounds: Identifying Markers that Predict Efficacy in Preventing Carcinogenesis

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Primary factors that can lead to ultraviolet radiation (UVR)-induced skin carcinogenesis are UVRinduced DNA damage, which leads to DNA mutations, and UVR-induced immunosuppression, which promotes skin tumour growth due to reductions in immune surveillance. The active metabolite of vitamin D, 1,25-dihydroxyvtamin D_3 (1,25D) has been shown to decrease UVR-induced DNA damage, immunosuppression and skin tumours. These effects were also observed with another vitamin D analog, 1,25-dihydroxylumisterol$_3$ (JN) and a vitamin D-like compound,

tetrahydrocurcumin (THC). It has long been considered that agents capable of reducing DNA damage and immunosuppression in acute UVR studies would effectively reduce skin carcinogenesis in chronic UVR models. However, the hybrid analog of 1,25D, 1α-hydroxymethyl-16-ene-24,24-difluoro-25hydroxy-26,27-bis-homovitaminD_3 (QW), as well as several other analogs, decreased UVR-induced DNA damage and immunosuppression, but did not inhibit skin carcinogenesis in a 40 week long murine photocarcinogenesis model. Therefore, our studies show that reductions in UVR-induced DNA damage and immune suppression alone are not necessarily predictive of potential for photoprotective agents to prevent skin carcinogenesis in the long term, and thus, there is a need to find more reliable markers. Phosphatase and tensin homolog (PTEN) and N-myc downstream regulated gene-1 (NDRG1) are tumour suppressor proteins that are reduced in some cancers and in metastasis respectively. Our studies have shown PTEN and NDRG1 are significantly reduced in human skin cells and Skh:hr1 mouse skin, following UVR exposure. Additionally, we have shown treatment with 1,25D immediately following UVR exposure significantly increased both PTEN and NDRG1 levels in primary human melanocytes and dermal fibroblasts. Phosphorylation of cyclic AMP response element binding protein (CREB) has been shown to increase following UVR and is linked to carcinogenesis. Our data illustrate that pCREB levels are significantly reduced by 1,25D treatment in Skh:hr1 mouse skin and human dermal fibroblasts after UVR. In addition, JN treatment of Skh:hr1 mouse skin and human dermal fibroblasts also reduced pCREB. The cytokines interleukin 6 (IL-6) and interleukin 10 (IL-10) have been shown in previous studies to promote the tumour microenvironment in skin cancers. Preliminary data from our studies have shown UVR exposure reduces IL-6 and IL-10 expression. We have also demonstrated that 1,25D can reduce IL-6 levels in mouse skin following UVR, and our preliminary studies indicated a similar trend for IL-10. Our studies have revealed several candidate biomarkers from acute UVR that may indicate whether photoprotective agents are likely to reduce tumours in a long term photocarcinogenesis model. This would make for a more time- and costefficient process for identifying suitable agents for this lengthy testing.

A multi-omic approach to understand solar exposure impact on skin ecosystem and evaluate a new SPF50+ sunscreen efficacy

Carine Jacques[1], Daniel Bacqueville[1], Emilien L. Jamin[2,3], Martine Maitre[1], Anais Noustens[1], Aimée Simcic Mori[1], Cécile Delsol[4], Laurent Debrauwer[2,3], Sandrine Bessou-Touya[1] & Hélène Duplan[1]

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Skin is the primary interface with the external environment, the skin ecosystem consisting of our microbiota, a collection of micro-organisms such as bacteria, viruses, and fungi reflect the state of the surrounding skin ecosystem. To evaluate the impact of external factors like sun exposure, the cutaneous ecosystem, that are skin, hydrolipidic film and microbiome, need to be studied as a whole to understand how they interact with each other and contribute to reply to external stress like sun exposure.
We developed a reconstructed human epidermal model (RHE) colonized with human microbiota and sebum to reproduce the complexity of the skin ecosystem. The RHE model was exposed to simulated solar radiation (SSR) with or without SPF50+ sunscreen (with UVB, UVA, long-UVA and visible light protection). A new SPF50+ photoprotective system containing a specific combination of four sun filters (TriAsorB, Tinosorb S, Uvinul T150 and Uvinul A+) and affording a broad spectrum UVB+A photoprotection, was evaluated on this *in vitro* model. Metabolomic profiles were performed by NMR and UHPLC-HRMS and lipidomic profiles by UHPLC-HRMS. In order to grant spatial information, mass spectrometry imaging (MSI) was used on the skin ecosystem model to investigate both composition and spatial distribution of diverse molecular species. Metagenomic analyses were performed from genomic DNA extract samples. ITS1 of the ribosomal RNA gene, and of the V1-V3 region of 16S gene were sequenced by high-speed sequencing.
Over 50 metabolites were significantly altered by SSR ($p<0.05$, log 2 values) with 8 main pathways affected, showing high skin oxidative stress and altered skin microbiota (branched-chain amino acid cycle and tryptophan pathway). Indeed, metabolomic analyses revealed high skin oxidative stress with modification of glutathione and purine pathway but also urea cycle. Other pathways have been highlighted linked to skin microbiota, like BCAAs cycle, lipid metabolism and tryptophan pathway. 16S and ITS rRNA sequencing showed the relative abundance of various bacteria and fungi were altered after SSR exposure. Major lipids pathways were modulated by SSR and correlate to MSI image giving spatial localization of the bioactive lipid molecules. Application of SPF50+ sunscreen to the *in vitro* model before exposure to SSR preserved the physiological interactions within the skin ecosystem and protected from sun exposure deleterious effects. These interactions are key parameters to avoid DNA damage, inflammation and immune suppression which play crucial roles in skin carcinogenesis. This study identified a highly accurate metabolomic signature of sun exposed skin using an *in vitro* model representative of the complete skin ecosystem. Global metabolomic signature was correlated to metagenomic analysis of skin microbiota and explain interactions between skin and microbiota.

Emergent PS in PDT

Invited speakers:

IL24 Bruno Therrien (Neuchatel, Switzerland)

Ruthenium Complexes in Photo-Dynamic Therapy

IL25 Vincent Sol (Limoges, France)

Synthesis, characterizations and properties of new phenalenone derivatives for photo-antibacterial activities and drug delivery.

IL26 Stéphanie Lhez (Limoges, France)

Free base porphyrin-cyanine dye conjugate: synthesis and optical properties

IL27 Enrico Caruso (Varese, Italy)

BODIPY: an emerging class of new photosensitizers Oral communications:

OC14 Sarah Haywood-Small: Water-Soluble Truncated Fatty Acid-Porphyrin Conjugates Provide Photo-Sensitiser Activity for Photodynamic Therapy in Malignant Mesothelioma: 2D versus 3D cell culture conditions.

OC15 Eurico Lima: Dansylpiperazino-bearing squaraines: an open door to new molecules with phototherapeutic interest

OC16 Vicente Marchán: Novel photosensitizers for combating hypoxic tumors

OC17 Cyrille Monnereau: Small distorted Pi-conjugated molecules as emerging photosensitizers in PDT

OC18 Odrun Gederaas: Pharmacokinetics of disulphonated tetraphenyl chlorin (fimaporfin/TPCS2a) in a rat glioma model

OC19 Alejandro Prieto Castañeda: New approaches on BODIPY-based photosensitizers: Solutions to dark toxicity towards the improvement of photodynamic therapy and phototheragnosis

Ruthenium Complexes in Photo-Dynamic Therapy

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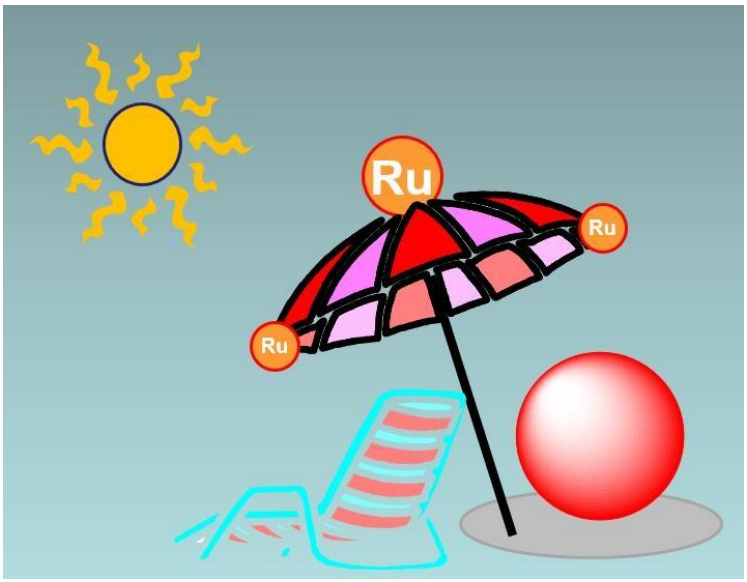
Porphyrin derivatives are known to absorb light and they are often used as photosensitizing agents in light harvesting processes or photo-dynamic therapy (PDT) treatments. Despite being used in the clinic to cure cancer, these so called first and second generations of porphyrin-based photosensitizers are limited by hydrophobicity, aggregation, photobleaching and slow clearance [1]. Therefore, in recent years, new photosensitizers have emerged to overcome these limitations. Of particular interest, those built around ruthenium complexes. The electronic and coordination properties of ruthenium provide several favorable characteristics to develop a new generation of photosensitizers. The rich photochemistry of ruthenium is well known and nicely exploited in light harvesting technology [2]. However, for biological applications such as PDT, the use of ruthenium-based photosensitizers remains in its infancy. During this presentation, recent results will be presented, showing the relevance of ruthenium complexes in PDT.



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Synthesis, characterizations and properties of new phenalenone derivatives for photo-antibacterial activities and drug delivery.

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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.[7]

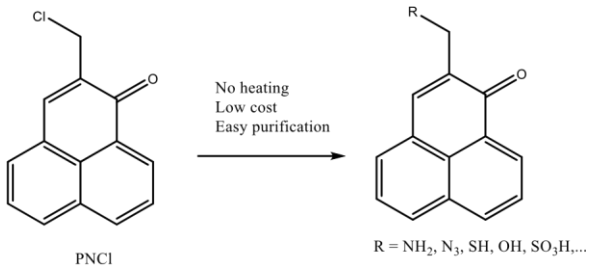

***Figure**: Examples of phenalenone functionnalization*

Different compounds are grafted on the phenalenone moieties in order to target selectively bacteria with oligosaccharide, cationic functions[8]…, with peptide to target organ cells or with fatty acids for made vesicles [9]. Phenalenone derivatives are also grafted on different materials as sand for decontamination of water[10].

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Free base porphyrin-cyanine dye conjugate: synthesis and optical properties

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Triplet formation by charge recombination[1] is a phenomenon that is encountered in many fields of the photo-sciences and can be a detrimental unwanted side effect, but can also be exploited as a useful triplet generation method, for instance in photodynamic therapy. In this framework, we will report the synthesis, characterization and photophysical properties of a novel free base porphyrin-cyanine conjugate based on the covalent combination of a cyanine dye with a tetraphenyl porphyrin unit. This novel type of porphyrincyanine photosensitizer has the ability to produce singlet oxygen in tetrahydrofuran and absorbs light at NIR wavelengths.[2]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement no. 764837 (Polythea project[3]).

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BODIPY: an emerging class of new photosensitizers

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Photodynamic therapy (PDT) is a therapeutic option based on systemic or topical administration of a non-toxic molecule, called photosensitizer (PS), followed by irradiation with a light of the desired wavelength. Generally, an incubation time is required between these two steps. Upon activation, the PS switches from its ground state to an excited singlet state, initiating a chain of electronic transitions that results in the production of death-inducing reactive oxygen species (ROS), mainly concerning singlet oxygen ($^1O_2$). This treatment can be used as an alternative to the most common antitumor therapies or as antibacterial treatment (in this case it is called aPDT).

There are different classes of photosensitizers, porphyrins and their derivatives, phthalocyanines, naphthalocyanines, but in recent years the scientific community has been interested in a new class of molecules suitable for PDT, the borondipirromethene compound, named BODIPY (Fig. 1). The BODIPYs are compounds largely used as fluorescent dyes. These molecules, easily synthesized in "one pot" procedure, exhibit quite high extinction coefficients [1], high resistance to photobleaching [2] and high quantum efficiencies of fluorescence. This last property is unfavourable to ROS formation thus fluorescence must be inhibited following chemical structural modifications (iodination of the 2,6 positions), thus favouring the process of triplet state formation and finally the production of $^1O_2$ [3].

In this work, I present an overview of the application of BODIPYs in the field of anticancer and antibacterial PDT.

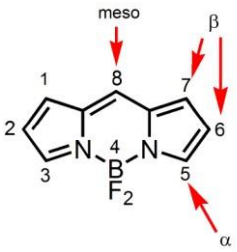

Fig. 1 Structure and specific position of the BODIPYs

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Water-Soluble Truncated Fatty Acid–Porphyrin Conjugates Provide Photo-Sensitiser Activity for Photodynamic Therapy in Malignant Mesothelioma: 2D versus 3D cell culture conditions.

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Objectives- Pleural mesothelioma is incurable. Intra-operative pleural photodynamic therapy (PDT) has recently emerged as a promising option to improve the multimodal treatment outcomes. The search for new, improved photosensitisers (PS) is ongoing, especially as PS have now entered clinical trials for mesothelioma. Few results have been published to date, although prolonged photosensitivity and a lack of tumour selectivity may limit the curative potential. Therefore, the focus of this approach is to develop and validate a series of water-soluble truncated fatty acid porphyrin conjugates, to assess if these agents can selectively kill mesothelioma cells in 2D and 3D culture models.

Method- The PDT effect was assessed in the human MSTO-211H and NCI-H28 mesothelioma cell lines, alongside a non-cancerous human mesothelial cell line, Met-5a. Cell viability was initially determined using the WST-8 cytotoxicity assay and then confirmed using the CellTiter-Glo® cell viability assay for 2D and 3D cultures. In some experiments, CellBrite cytoplasmic membrane dyes were used to label cell lines within a co-culture system (mesothelioma cell lines were cultured with either MRC-5 fibroblasts, THP-1 monocytes or the Jurkat T lymphocyte cell line). 3D spheroids were also generated in some conditions using ultra-low attachment plates. Flow cytometry of Annexin VFITC/PI-stained cells confirmed the induction cell death.

Results- Herein, we report the synthesis, photophysical, and photobiological properties of four porphyrin-based PS conjugated to truncated fatty acids (C5SHU to C8SHU). PS have been developed using a high content approach with high fluorescence intensity and singlet oxygen quantum yields. PS appeared to selectively localise within the non-nuclear compartments of cells before exhibiting high phototoxicity. Initial studies indicate that there is a large decrease in cell viability 24h post treatment with C5SHU and C6SHU PS coupled with 15-minute irradiation using red LEDs, both apoptosis and necrosis were induced at 24 and 48 h. Currents studies are focussing on using longer exposure times of up to an hour (the standard clinical exposure time for intraoperative PDT) in addition to gathering data from the 3D and coculture systems to further assess PDT drug responses.

Conclusions- As our pentanoic acid-derivatised porphyrin (C5SHU) induced the largest anti-tumor effect in this study in 2D conditions, we put this forward as an anti-tumour drug candidate in PDT and photo-imaging diagnosis in mesothelioma. Nevertheless, the 3D co-cultures appear to be a more realistic tumour microenvironment for PDT drug responses, when compared to 2D cell models, and thus are superior preclinical cancer models. Future directions will build on the developments, aiming to refine these porphyrin-based PS clinically towards mesothelioma diagnosis and PDT.

Dansylpiperazino-bearing squaraines: an open door to new molecules with phototherapeutic interest

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In 1965, Treibs and Jacob prepared the first squaraine by reacting squaric acid with an aromatic heterocyclic methylene base, without knowing the panoply of dyes that would be designed from theirs. For photodynamic therapy (PDT), these dyes gained a particular interest in research since they display intense absorption bands in the region of the electromagnetic spectrum where tissues are more transparent to light (650-850 nm), allowing to overcome the most significant deficiency of porphyrins, a family of dyes whose clinically applied photosensitizers currently belong mainly.

This communication presents the synthesis of three *N*-hexyl squaraine dyes, derived from heterocycles of different natures (benzothiazole, indolenine and benz[*e*]indole), functionalized in the central fourmembered ring with the dansylpiperazino group. After their complete structural characterization, the stability of the dyes to light was evaluated, as well as their ability to form singlet oxygen using the 1,3diphenylisobenzofuran (DPBF) assay. Then, for an initial screening, half-maximal inhibitory concentration values (IC_{50}) of the dyes were determined against two cell lines, a prostate tumor cell line (PC-3) and a normal cell line (NHDF), using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. Based on MTT data, phototoxicity and tumor selectivity ratios were calculated. Then, selecting the dye with the most significant therapeutic interest, its mechanism of action was studied, determining the preferential organelle where they accumulate inside cells, the reactive oxygen species that are involved in their photodynamic activity, cell cycle arrest studies and assessing the activation of caspase-3 and -7, both activated during apoptosis cell death.

The results highlight the relevance of the potential phototherapeutic application of squaraine dyes, unexpectedly standing out among the prepared dansylpiperazino-bearing benzothiazole derivative. Furthermore, this dye showed high phototoxicity and tumor selectivity ratios, presenting, among other photophysicochemical and photobiological properties, several characteristics inherent to those of an ideal photosensitizer.

Novel photosensitizers for combating hypoxic tumors

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Photodynamic therapy (PDT) is a well-established, non-invasive modality for the destruction of tumors and/or tumor vasculature, based on the combination of three components, namely: a photosensitizer (PS), light of suitable wavelength, and oxygen. However, PDT is less efficient in the treatment of deepseated hypoxic tumors, because it is an oxygen-dependent process. Here we introduce a new family of PSs that take advantage of the well-established anticancer properties of transition metal complexes and of the rich and tunable photophysical properties of organic fluorophores. Phototoxicity studies have revealed that the PSs are non-toxic in the dark but become highly phototoxic when irradiated at different wavelengths within the phototherapeutic window, even with highly-penetrating far-red and NIR light. In general, the new PSs exhibited IC_{50} values in the very low nM range (e.g., 7.4 nM at 645 nm) and impressive PI values (PI > 34000) after red light irradiation. In addition, the PSs display a good phototoxicity profile with highly penetrating NIR light (e.g., IC_{50} = 0.26 □M at 770 nm), and retain an excellent photoactivity under hypoxia. Finally, it is worth noting that the PSs are aqueous-soluble and can be prepared in high purity from straightforward syntheses, which are also highly desirable attributes for further development. Thus, the newly developed PSs are promising candidates for treatment of large and deep-seated hypoxic tumors, which is the cornerstone of PDT.

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Small distorted π-conjugated molecules as emerging photosensitizers in PDT

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PhotoDynamic Therapy (PDT) relies on the use of light in conjunction with a photoactive molecule, called photosensitizer, to promote cell death upon photoirradiation, generally by accumulation within the cell of Reactive Oxygen Species (ROS). Although used clinically for decades for treating a variety of cancer, current treatments are mostly based on porphynoid molecules, which present a relatively narrow therapeutic range (*ie* a significant dark cytotoxicity). Alternatives based on small molecules often involve heavy atoms (transition metals or halogen) which may also be a concern for long time toxicity. In our group we have been developing approaches based small, highly distorted π-conjugated molecules that present large singlet-oxygen generation efficiencies. [1, 2]

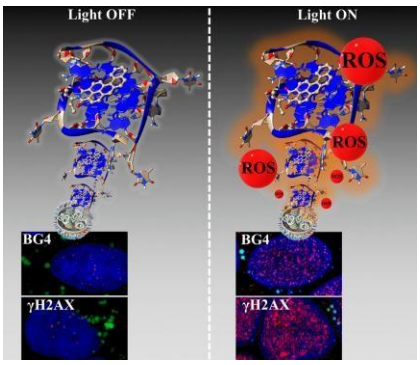

Figure 1. Principle of action of DBI within two cancer cell lines. Oxydative damages post-irradiation seen as red spots

In particular, for one of these molecules (**DBI**, Figure 1), good cell internalization and highly efficient photoinduced cell death (10 nm range) could be achieved, with minimal dark toxicity.[3] Associated mechanisms, based on accumulation within exosomes and G4 targeting, will be discussed in this talk, along with strategies towards optimization of their spectroscopic properties (absorbance in the NIR).

Reference

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Pharmacokinetics of disulphonated tetraphenyl chlorin (fimaporfin/$TPCS_{2a}$) in a rat glioma model

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Glioblastoma multiforme (GMB) is one of the most aggressive cancers in human, with low cure rates for many cancers after surgery, ionizing radiation or chemotherapy. Because of that, the interest in development of new treatment technologies and using alternative substances is continuously increasing.

The photosensitizing potential of the photosensitizer meso-tetraphenyl chlorin disulphonate (fimaporfin/$TPCS_{2a}$, Amphinex®) has been evaluated by using a rat orthotopic glioma model, 10 days after intracranial instillation of F98 cells in hippocampus of Fisher 344 rats. The injection of fimaporfin (3 mg/200 g rat) was performed in one of the tail veins of the tumor-bearing animals and followed by in vivo fluorescence imaging, whole heads, bodies, and ex vivo analyses of glioma tumors and blood samples. The tumor localization and size in hippocampus were documented by MRI 9 days after F98 cell instillation and the results indicates no fluorescence in the blood samples post 6 days, neither from the intravenous nor subcutaneous injections. Interestingly, there is clear fluorescence in the glioma ex vivo analyses 18 days after F98 cell instillation in the hippocampus (10 days post PDT), which shows a fimaporfin accumulation in gliomas much longer than in the circulating blood.

New approaches on bodipy-based photosensitizers: solutions to dark toxicity towards the improvement of photodynamic therapy and phototheragnosis

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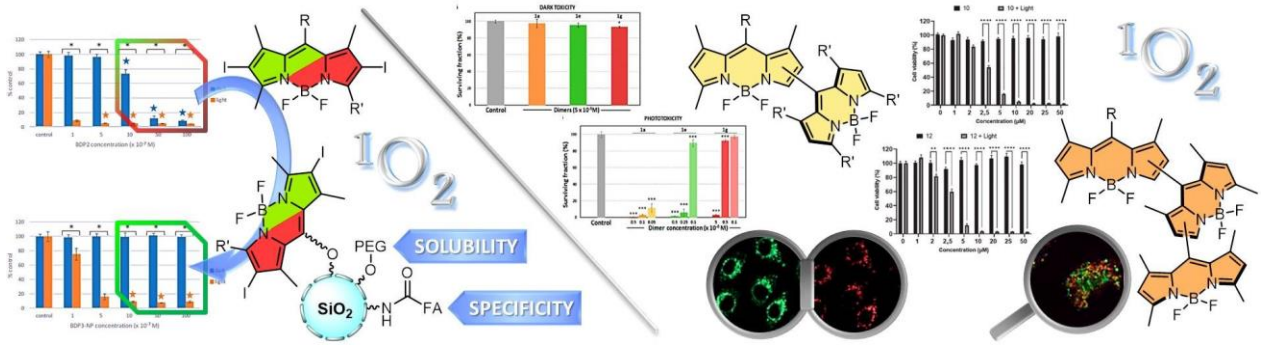
The development of new agents for photodynamic therapy (PDT) is an active research area due to the high interest in establishing non-invasive cancer treatments. However, developing novel and effective PDT agents (*i.e.* photosensitizers, PSs) is not trivial and requires a fine balance among multiple properties.[1] BODIPY-based PSs are a well-established family because of the chemical versatility of the BODIPY chromophore.[2] The generation of $^1O_2$ is traditionally achieved by iodinated and brominated BODIPYs, but they have poor photostability and show high dark toxicity, reducing their usefulness as PSs. To address these limitations, two approaches can be followed: 1) use of nanomaterials as (photo)drug carriers, increasing selectivity and biocompatibility,[3] and 2) design of halogen-free PSs by direct linkage of BODIPYs orthogonally arranged.[4] Here we present a series of tailor-made halogenBODIPY-containing nanoparticles and BODIPY dimers/trimers.[5] They are designed to overcome dark toxicity, enhance their application in PDT, and could inspire novel phototheragnostic agents.



BODIPY-based PSs: halogenBODIPY nanoparticles and BODIPY dimers/trimers

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Communications in health and skin photobiology

Oral communications:

OC20 Huang Chiao Huang: Photodynamic therapy overcomes ABC transporters-mediated multidrug resistance

OC21 Lise Aubry: Toxicological impact of photodegraded organic tattoo pigments on human cultured keratinocytes

OC22 Philipsen Peter: Assessment of candidate action spectra for vitamin D3 using a set of lamps with spectra of different qualities

OC23 Dietrich Averbeck: Focus on the importance of photoinduced effects on mitochondria and their biological and phototherapeutic consequences

OC24 Nan Gao: Development of peptide-based tools to measure cellular free heme after UVA irradiation

OC25 Francesco Garzella: Characterization of the GafChromicTM EBT film as 2D sensor for UV and blue-light dosimetry

OC26 Cyrus Kazemiraad: Metabolic Changes in Human Umbilical Vein Endothelial Cells (HUVEC) Induced by Photobiomodulation: A Fluorescence Lifetime Imaging Microscopy (FLIM)-Based Monitoring Approach

OC27 Thierry Douki: DNA photoproducts outside of the nucleus as biomarkers of the genotoxicity of UV radiation

OC28 Christopher Kremslehner: 3D human organotypic epidermal skin equivalents containing UVB-pretreated and labeled keratinocytes as a model system for multimodal analytical investigation of tissues

OC29 Roger Bresoli-Obach: NIR/SWIR microscope: a new approach for imaging biologically relevant samples

OC30 Celina Pihl: Oral supplementation with quercetin and fisetin potentiates photocarcinogenesis in UVR exposed hairless mice

Photodynamic therapy overcomes ABC transporters-mediated multidrug resistance

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One of the major problems encountered in cancer treatment is the development of multidrug resistance, a process which decreases the efficiency of chemotherapy in cancer patients. Among the 48 human ABC proteins , P-glycoprotein (P-gp, ABCB1) and breast cancer resistance protein (BRP1, ABCG2) have been shown to play an important role in the development of MDR. These transporters utilize energy from ATPbinding and hydrolysis for the efflux of a number of chemically dissimilar hydrophobic or amphiphathic anticancer agents. To date, the development of ABC-drug transporter inhibitors to sensitize cancer cells to chemotherapy has been unsuccessful in the clinic. This is because some ABC transporter inhibitors are too toxic, while others induce pharmacokinetic changes due to drug-drug interactions. Photodynamic therapy (PDT) is a non-thermal, photochemistry-based modality that involves harmless red-light activation of photosensitizers to generated short-lived, cytotoxic reactive oxygen species (ROS). Based on our recent discovery that FDA-approved benzoporphyrin derivative (BPD) photosensitizer is a substrate for both P-gp and ABCG2, we hypothesize that light activation of BPD can selectively block the function of P-gp and ABCG2. Using P-gp or ABCG2 expressing total membrane vesicles of High-five cells, we have shown that light activation of BPD resulted in increased inhibition of the ATPase activity of P-gp and ABCG2. Gel electrophoresis and Western blotting further confirmed that PDT induces the aggregation of P-gp and ABCG2 by covalent crosslinking. Our studies provided the proof of principle that PDT can inhibit the function of ABC drug transporters by modulating their ATPase activity and protein integrity, which can aid in the development of new tools to overcome cancer multidrug resistance.

Toxicological impact of photodegraded organic tattoo pigments on human cultured keratinocytes

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Tattooing is very popular around the world. In spite of the large population concerned, the safety of tattoos remains to be fully evaluated. A major issue is the pigments, which are insoluble compounds that persist in the skin dermis for years. Organic pigments, including azo and polycyclic pigments, are increasingly used in tattoo inks. Once in the dermis, these compounds may be altered by sun exposure or dermic macrophage activity. These processes may generate soluble molecules, which could diffuse into the epidermis and affect the major cells of this layer, keratinocytes. Interestingly, a link is suspected between tattooed skin and some cutaneous diseases like basal-cell carcinoma. Only few articles deal with the toxicity of degraded pigments in the epidermis. This topic is the basis of ongoing work in our group.

In vitro studies using the HaCaT keratinocytic cell line allowed us to unravel the effects of photodegradation on the toxicological profile (viability, oxidative stress) of a series of organic pigments. Ageing was simulated with a test chamber (Q-SUN Xe-1 Xenon) combining sunlight exposure and increased temperature (40°C). First, biological results show different cytotoxic profile between a series of pristine organic pigments and suspensions exposed to heat and simulated sunlight. For some pigments, ageing was found to modulate the toxicological endpoints investigated.

Emphasis was placed on the azo orange pigment, Po13. HaCaT viability was significantly decreased after 24-hour exposure with 0.3 µg/mL of pristine or aged Po13 and no difference with sunlight ageing was noticed. In both conditions, Po13 could not generate any detectable reactive oxygen species in HaCaT. Interestingly, high-performance liquid chromatography coupled to mass spectrometry showed that Po13 was sensitive to sunlight. Its major photoproduct could be identified and isolated for evaluation of its toxicity. Moreover, 96-hour sunlight exposure seems to induce reduction of the mean hydrodynamic diameter of Po13 particles.

Further studies will investigate the induction of DNA breaks, the toxicological profile of Po13 aged by simulated sunlight for 96 hours, and the ageing with a solution mimicking the lysosomic condition in macrophages. Proteomics will study the effects of degraded pigments in keratinocyte protein expression.

Assessment of candidate action spectra for vitamin D3 using a set of lamps with spectra of different qualities

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Current public health advice on optimal vitamin D status maintenance is (partly) based on scenario modeling of the photoproduction of vitamin D. Such modeling relies on the CIE action spectrum for previtamin D production, which is under debate. Young et al, (2021) have exposed sets of healthy young volunteers to UV-lamps with spectra of different qualities and logged their resulting vitamin D status by measuring serum 25(OH)D. With this set of measurements, they showed that a blue-shifted version of the CIE action spectrum better explains the measurements than the original. This observation can have consequences for the advice to the public and calls for a broader assessment. In this presentation, we will exploit the same set of measurements as collected by Young et al to compare the performance of many of the currently available proposed action spectra for vitamin D production. These include the original CIE, shifted CIE (by varying degrees), Bolsee, (modified) QUT and RIVM (for different skin sites, see Van Dijk, 2016). We compare both full body exposure and partial body exposure. We have found that, for 25(OH)D measurements, some of the RIVM action spectra are superior. We will discuss if it is time to replace the CIE action spectrum with a new standard.

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Focus on the importance of photoinduced effects on mitochondria and their biological and phototherapeutic consequences

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50 years ago, fundamental research was focused on genotoxic effects photoinduced by short wave UV (UVC) and solar UV radiation. This involved important research efforts and discoveries on the photolesions induced in DNA, the mechanisms of repair in eukaryotic cells (yeast and mammalian cells) and mutagenic and cancerous effects. In parallel, there was growing interest in photodynamic anticancer effects (involving singlet oxygen production) and also in the photoinduced binding of photosensitizers such as furocoumarins (psoralen derivatives) and their beneficial photodermatological effects on skin diseases. Interestingly, the combination of psoralens derivatives with UVA (PUVA) was shown to be quite effective in the treatment of psoriasis. So-called bifunctional psoralens such as 8-methoxypsoralen (8MOP) and Bergapten (5-methoxypsoralen, 5-MOP) photoinducing monoadducts- and biadducts (interstrand-crosslinks) in DNA could slow down the high proliferation rate of psoriatic cells. In the following, it could be demonstrated that also mono-functional, i. e. monoadduct forming furocoumarins plus UVA and also UVB alone could be very effective but with less side-effects. Very intriguingly, in eukaryotic cells these treatments were very efficiently inducing damage to mitochondria but less to nuclear DNA. In fact, there is much more to the mitochondrial activity than just its well-known activity in cellular energy metabolism. The present overview retraces the evidence demonstrating the importance of mitochondria in cellular immune responses with focus on the photobiological and beneficial phototherapeutic consequences of UVB, photodynamic and photoreactive treatments.

Development of peptide-based tools to measure cellular free heme after UVA irradiation

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Heme plays a vital role in cell biology and the dysregulation of heme levels is implicated in a wide range of diseases. Currently however there is a lack of convenient chemical tools that can be used to measure the changes in heme levels in biological media or in live cells. Exposure of skin cells to solar UVA irradiation has been shown to result in elevated levels of free heme and upregulation of Heme oxygenase-1 (HO-1), the oxidant-inducible enzyme that catalyses the breakdown of pro-oxidant heme.[1] There is therefore considerable interest in developing new probe molecules to study such UVA-induced changes in heme levels and gain a greater understanding of the detrimental and potentially beneficial effects of UVA on human skin.

The aim of this work is to explore the development of probe molecules that are derived from natural heme-binding proteins. We will describe a tryptophan-containing peptide probe based on a peptide sequence from the natural heme-binding protein, Bach-1[2] a transcription factor that acts as a negative regulator of the HO-1 gene. The fluorescence of the tryptophan residue can be quenched upon binding of heme to the probe peptide providing a direct readout of heme levels. Here, we describe an efficient and scalable synthesis of the novel fluorescent tryptophan analogue, 7-aza-tryptophan that is incorporated into the Bach-1 derived peptide by solid phase peptide synthesis. This peptide probe can sensitively measure changes in heme levels in human dermal fibroblasts, FEK4 cells following exposure to increasing levels of UVA irradiation (up to 500 kJ/m^2).

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Characterization of the GafChromic™ EBT film as 2D sensor for UV and blue-light dosimetry

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With the rise of the Photodynamic Therapy (PDT) applications, the number and variety of the requested light sources has considerably increased. However, homogeneity of the emission is crucial for a correct PDT but is often not trivial[1]. The GAFChromic™ films (GCFs) are a family of self-developing materials, made by an active monomer layer, sandwiched by visible-light protective layers, that after interacting with electromagnetic radiation undergoes to polymerization process, reflecting in film's darkening[2]. Nowadays, while main GCFs applications include absolute[3] X-ray/gamma-ray dose measurements in the biomedical field, their intrinsic light-induced darkening opens the way to a fruitful application for the spatial characterization of light sources in the UV-Blue range[4]. Along this work, we measured the optical response and the lateral resolution of two GCF models (EBT-2 and EBT-3) and tested their application as a 2D dose sensor. In a writing/reading approach via confocal microscope, 405 nm light is used to induce the film darkening while a second wavelength in the range 405-633nm is used for reading in transmission mode. Measurements start from the definition of a response function relating the pixel value to the energy (mJ), crucial for GCFs calibration. Subsequently, the resolution of the film-confocal reading system was characterized by evaluating the response to a 405 nm laser beam tightly focused. Lastly, we applied GCFs to the characterization of both the irradiance and spatial homogeneity of exemplary light sources.

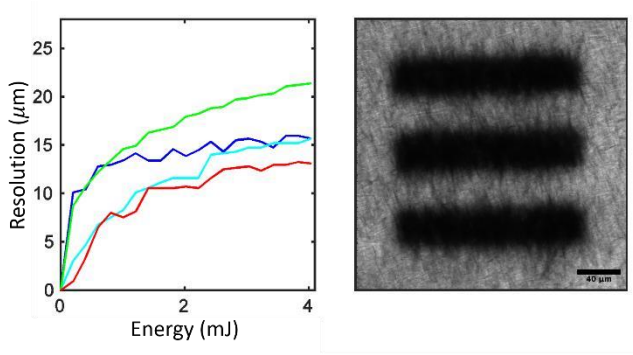

Figure 1. Left. The dependence of the space resolution from the energy at four different reading wavelengths (405 nm, Blue, 488 nm, Blue, 532 nm, green, and 633 nm, red) after writing with 405 nm. Right. Microscope imaging of GCF EBT response to darkening at 365 nm with a USAF 1951 clear path target in front.

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Metabolic Changes in Human Umbilical Vein Endothelial Cells (HUVEC) Induced by Photobiomodulation: A Fluorescence Lifetime Imaging Microscopy (FLIM)Based Monitoring Approach

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NADH and FAD are pivotal electron donors involved in glycolysis and oxidative phosphorylation (OXPHOS), with FAD primarily contributing to OXPHOS. The fluorescence characteristics of these coenzymes, which depend on their redox and binding states, offer potential for nondestructive monitoring of cellular metabolism. Simultaneous FLIM recording of NADH and FAD allows to spatially assess their fluorescence lifetimes and intensities, both in their free and protein-bound forms, which are closely linked to cellular metabolism.

Photobiomodulation (PBM) therapy utilizes specific wavelengths and sub-thermal radiometric conditions to facilitate tissue healing. However, the precise mechanisms by which PBM affects cellular metabolism and the optimal conditions for its applications are yet to be fully elucidated. The complex nature of PBM necessitates the exploration of numerous variables, rendering conventional assays challenging for determining optimal PBM parameters. The use of FLIMbased metabolic imaging presents an interesting opportunity for the investigation of PBM effects in quasi real-time, enabling rapid explorations of various PBM conditions.

In this study, we present the first application of FLIM to assess the metabolic effects of PBM on HUVEC cell line. Our findings reveal a notable increase in mitochondrial OXPHOS induced by PBM. This increase is revealed by the NADH and FLIRR metabolic indices, which are derived from the decomposition and processing of the multi-exponential fluorescence decays of NADH and FAD.

Overall, our results highlight the potential of FLIM as a valuable tool for studying cellular metabolism and evaluating the impact of PBM on HUVEC. This research contributes to a better understanding of the metabolic changes induced by PBM and paves the way for further investigations in the field of PBM therapy.

DNA photoproducts outside of the nucleus as biomarkers of the genotoxicity of UV radiation

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UV-induced formation of photoproducts in DNA is a major initiating event of skin cancer. Consequently, many analytical tools have been developed for their quantification in nuclear DNA. In the present work, we developed an assay for the quantification of the pyrimidine dimers in easily accessible biological fluids like urine for *in vivo* studies and cell culture medium for *in vitro* investigations. We targeted the DNA photoproducts present in the short oligonucleotides released by the nucleotide excision repair machinery. Unlike other approaches, our assay does not only quantify the dinucleotides expected to be released from these repair products by action of endogenous nucleases. Our technique combines isolation of the photoproducts-bearing oligonucleotides to enzymatic hydrolysis and HPLC-tandem mass spectrometry detection with isotopic dilution.

Biological validation of the approach was first obtained from experiments in HaCat keratinocytes. We could unambiguously follow the transfer of photoproducts first from nuclear DNA to cytoplasm, and in a second step from cytoplasm to culture medium. Release of pyrimidine dimers in the culture medium was also observed in dose- and time-dependent manners from reconstructed human epidermis and human skin explants. Relevance of the assay to human exposure was shown by the detection of pyrimidine dimers in the urine of volunteers collected after recreational exposure in summer. Experiments in RHE and a first clinical study showed that detection of DNA photoproducts in fluids could be a valuable tool in the assessment of photoprotection.

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3D human organotypic epidermal skin equivalents containing UVB-pretreated and labeled keratinocytes as a model system for multimodal analytical investigation of tissues

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The consequences of UVB exposure on the fate of epidermal keratinocytes are both important but also a well investigated topic for basic and clinical science. Typically, the damage, the biological response and the fate of cells have been investigated with histological and (bio)chemical techniques. Recently cooperative efforts have been initiated, to combine several state-of-the-art spatial analytical methods from the optical/chemical and physical fields to gain holistic information on tissues by "correlated multimodal imaging". In the course of the EU-COST action COMULIS (Correlated Multimodal Imaging in Life Sciences), our laboratory had the opportunity to provide a human organotypic epidermal skin equivalent model (SE) to a consortium of more than 15 labs in 11 countries with the aim to address the consequences of UVB irradiation at cellular level in the tissue context. The goal was on the one hand to assess the compatibility and order of analytical methods and on the other hand also to answer the basic science question on whether we could gain novel insights on the fate of UVB damaged cells in the tissue context. For this we generated a version of our SE where the epidermal part was spiked with 5% UVB pre-irradiated (20mj/cm²) and labelled keratinocytes. In this study we present results from spatial transcriptomic analysis (Visium, 10x Genomics) and oxidized lipid intensities from MALDI mass spectrometry imaging (MSI) localized to immunohistological markers with StrataQuest (TissueGnostics) context analysis software.

In brief, we could identify oxidized lipid signatures and transcriptional patterns associated with UVB and could localize these within the tissue accounting differentiation state, position within the microenvironment, and immunohistiological markers at cellular resolution. This new holistic analytical approach will yield insights into the UV-induced mechanisms underlying skin damage, aging and cancers, that would evade stand-alone analytical methods.

NIR/SWIR microscope: a new approach for imaging biologically relevant samples.

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Imaging with near- and short-wave infrared radiation (NIR and SWIR, respectively), enabled by recent technological breakthroughs in InGaAs optical detectors, is gaining relevance in the field of medical research due to SWIR's ability to penetrate deeper into soft tissue while minimizing the scattering and autofluorescence tissue contribution, thereby providing images with a superior quality.[1] Moreover, the energy of NIR/SWIR radiation is insufficient to trigger the photodynamic effect, which enhances the range of conditions were a biologically relevant structure can be imaged.[2] The development of a novel NIR/SWIR imaging device to image the NIR/SWIR emission from SWIR emitters and singlet oxygen phosphorescence ($^1O_2$) will be presented. The developed NIR/SWIR microscope prototype is inspired on a scanning confocal microscope, although no pinhole in the detection path is used to increase its sensitivity. The prototype has been validated to assess the signal linearity with the concentration, absorbance of the sample and the excitation power. The validation of the prototype has been performed monitoring the $^1O_2$ phosphorescence as an example of a very weak emitter ($F_P \approx 1x10^{-6}$ in water). Once validated, images from different relevant biological samples, such as the PpIX phosphorescence of a pine tree leaf or the $^1O_2$ phosphorescence from a photosensitizer dyed cotton fibber, have been acquired (see Figure 1).

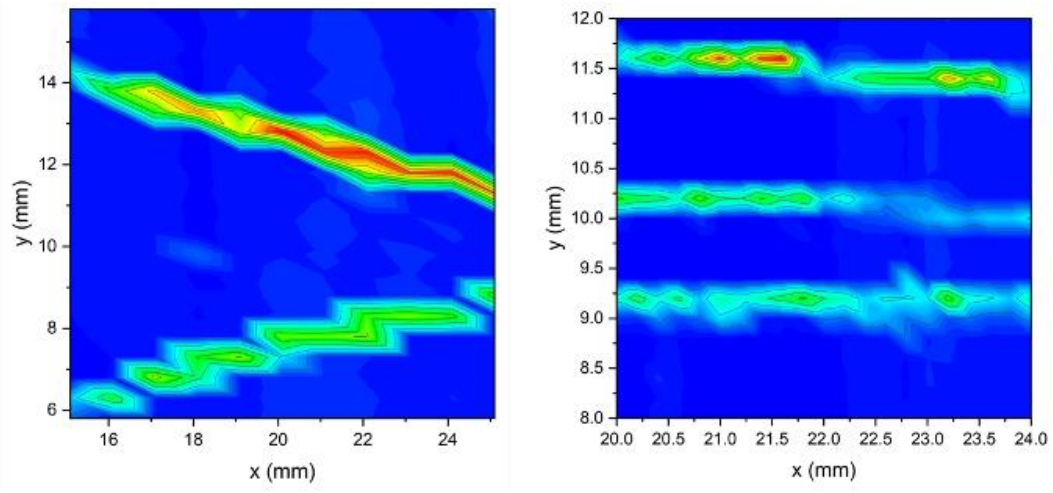

Figure 1 *Proof-of-concept NIR/SWIR images. Left: PpIX phosphorescence from two pine tree leaves. Right: $^1O_2$ phosphorescence from a phenalenone-dyed cotton fibber.*

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Oral supplementation with quercetin and fisetin potentiates photocarcinogenesis in UVR exposed hairless mice

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Background/aim: Quercetin is a polyphenolic compound found ubiquitously in nature. Quercetin has demonstrated anticarcinogenic properties against several cancer cell lines and is reported to protect against ultraviolet radiation (UVR) induced inflammation and oxidative stress in hairless mice. Structurally related compounds fisetin and rutin have demonstrated comparable effects, indicating a similar photoprotective potential for the three compounds. Here, we evaluated the abilities of oral supplementation of quercetin, fisetin, and rutin to protect against photocarcinogenesis in hairless mice. *Materials and methods:* Female hairless C3.Cg-Hrhr/TifBom Tac mice (n=125) underwent thrice-weekly exposure to UVR equivalent to three standard erythema doses to induce photocarcinogenesis. Oral supplementation with the three compounds began two weeks prior to the start of UVR protocol. The animals received either 100 mg/kg quercetin, fisetin, or rutin in the feed, 600 mg/kg nicotinamide in water as a positive control, or no supplementation acting as the UV control. *Results:* Oral supplementation with quercetin reduced time to tumour onset of the second and third squamous cell carcinoma in hairless mice compared to the UV control (183 [95% CI: 171, 192] vs 192 days [95% CI: 183, 203] and 183 [95% CI: 183, 192] vs 203 [95% CI: 203, 217], respectively). Oral supplementation with fisetin reduced time until the third tumour (203 [95% CI: 192, 203] vs 203 days [95% CI: 203, 217]), with no effect of rutin supplementation. Quercetin supplementation was associated with an increase in tumour growth, while fisetin increased UVR-induced oedema formation. The compounds demonstrated no accumulation in the skin evaluated through mass-spectrometry imaging. *Conclusion:* Oral supplementation with quercetin and fisetin potentiates UVR-induced photocarcinogenesis in hairless mice, indicating a need for caution when selecting appropriate photoprotectants.

Flash presentations in PDT

Flash presentations (see poster sessions for abstracts):

Maria Tesa: Combining Photoluminescence and Transient Absorption Spectroscopy for Photodynamic Therapy Research

Laura Espinar: A Type-I Photosensitiser for Hypoxic Photodynamic Therapy

Ester D'agostino: Synthesis and connection of two chromophores: a promising Glyco-OPE-Porphirin and its aggregation properties.

Pietro Bertolotti: Membrane potential modulation in bacteria via push-pull azobenzene

Mine Demir: 5-ALA Mediated PDT/Radiotherapy Combination for Breast Cancer Using Gold Chalcogenides

Andreas Fellner: Fly into the light: Treating fruit fly Drosophila melanogaster with Photodynamic Inactivation based on Na-Mg-chlorophyllin

Beata Kruszewska-Naczk: Genes encoding proteins engaged in DNA repair are important in the response of bacteria to antimicrobial Blue Light

Linda Jernej: Photodynamic Inactivation in Agriculture: Combating Fungal Phytopathogens Resistant to Conventional Treatment

Martina Mušković: Amphiphilic cationic tripyridiniumporphyrins and their Zn(II) complexes: the influence of the irradiation wavelength and the length of the alkyl chain

Maria Alexandra Cucu: Designing in vitro aPDT experiments from A to Z by optical methods only

Maria Bartolomeu: A study of Wastewater Disinfection with Photodynamic Treatment and its Ecotoxicological
Effects

Intact organisms and photosynthetic biohybrids

Invited speakers:

IL28 Massimo Trotta (Bari, Italy)

The mighty power of photosynthesis

IL29 Matteo Grattieri (Bari, Itaky)

The Biotic/Abiotic Interface in Photosynthetic Bacteria-Based Biohybrid Electrochemical Systems

IL30 Melania Reggente: (Lausanne, Switzerland)

Bioengineering anodes for biophotovoltaic (BPV) applications

IL31 Rossella Labarile (Bari, Italy)

In situ polydopamine coating for enhanced electron transfer from photosynthetic bacteria

IL32 Noam Adir (Haifa, Israel)

Live Cell Based Bio-Photoelectrochemical Cells for Clean Solar Energy Conversion

Oral communications:

OC31 Pierluigi Lasala: Enhancing microbial biophotoanodes efficiency with functionalized gold nanostructures

OC32 Romain Rouxel: Light-harvesting processes in green sulfur bacteria in vivo

OC33 Nikolay Ryzhkov: Electric polarization effect on photosynthetic efficiency, non-photochemical quenching and photovoltaic performance of intact Limnospira indica based photoelectrodes

OC34 Cesar Vicente-Garcia: Diatom Microalgae: Overlooked Candidates for the Construction of Robust Biophotovoltaic Devices

The mighty power of photosynthesis

Massimo Trotta [1]

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The Biotic/Abiotic Interface in Photosynthetic Bacteria-Based Biohybrid Electrochemical Systems

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Utilizing anoxygenic photosynthetic bacteria to develop biohybrid electrochemical systems is very attractive due to the versatile metabolism of these organisms that enables obtaining light-driven biosensors, power generators, and bioelectrofactories. However, the insulating nature of the bacterial outer membrane and the peptidoglycan layer surrounding the photosynthetic machinery make the harvesting of photo-induced electrons challenging.[1] Accordingly, various artificial approaches have been reported to facilitate this extracellular electron transfer, and our research efforts have been focused on tuning the biotic/abiotic interface using a multiscale approach. We developed a polydopamine-purple bacteria redox-adhesive matrix that enhanced biophotocurrent generation thanks to the capability of quinones to interact with the photosynthetic apparatus, achieving a 5-fold increase in current density.[2] We then employed home-made, bio-based, porous electrodes based on polyhydroxybutyrate with the redox-adhesive biohybrid matrix, allowing an additional increase in biophotocurrent. The possibility to implement the obtained biohybrid system for the light-driven monitoring of organic contaminants will be presented,[3] and the challenges and outlook of this exciting research field will be discussed.

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Bioengineering anodes for biophotovoltaic (BPV) applications

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Extracellular electron conduits are pathways by which microbes can electronically communicate with their environments. Since their discovery, researchers have focused on developing new technologies that exploit these conduits for environmental and energy applications. However, except for a limited number of natural exoelectrogens, most microbes possess insulating membranes that impair efficient extracellular electron transfer.

This presentation focuses on emerging methods for enhancing microbial charge extraction from lightharvesting cyanobacteria. Through nanoparticle engineering, we developed an approach for concomitantly monitoring cyanobacteria division while enhancing its ability to transfer charge to an electrode. We show passive, length-dependent internalization of synthetic nanoparticles under conditions that do not impair the photosynthetic activity of the cells. When interfaced with an electrode, the nanobionic cells show a 15-fold enhancement in photocurrent compared to unmodified cells in the absence of the nanoparticles. This enhancement is attributed to the nanoparticle-enabled, cross-membrane bridging of the cells to the electrode.

This technology lays the framework for next-generation microbial electronics based on biosynthetizable nanoparticles. We further demonstrate the application of a biosynthetic polydopamine (PDA) nanoparticle coating on the outer membrane of the photosynthetic organism *Synechocystis* sp. PCC6803. The resulting conductive shell comprises PDA nanoparticles that enhance electrode adhesion and improve microbial charge extraction. A combination of scanning electron microscopy (SEM), transmission electron microscopy (TEM), UV-Vis absorption, and Raman spectroscopy measurements was used to characterize the nanoparticle shell at varying synthesis conditions. Under optimized conditions, we show sustained cell growth and observe a third-fold enhancement of photocurrent from the PDAcoated cells compared to unmodified cells. This study paves the way for new cell microbial electrodes based on inter and intra-cellular conductive networks that are biologically synthesized.

In situ polydopamine coating for enhanced electron transfer from photosynthetic bacteria

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Bioelectronics promise fundamental advances in the sustainable production of energy thanks to the possibility to couple microorganisms with electronic components in biohybrid devices. The optimization of the interface between biotic and abiotic components can be achieved through the *in situ* coating[1, 2] of the membrane of metabolically active cells of electrochemically active bacteria. Polydopamine (PDA) is a bio-inspired polymer produced by the self-polymerization of dopamine[3] in mild and biocompatible conditions able to coat the cells and act as a soft functional matrix to optimize the bio-hybrid interface.

Here, the *in situ* polymerization of dopamine was exploited to develop a bio-hybrid systems based on the unique characteristics of the photosynthetic purple non sulfur bacterium *Rhodobacter* (*R.*) *sphaeroides* to thrive using light. Electrochemical characterization was performed to analyze the electronic behavior of these biohybrids, unveiling that the polymer layer on the bacterial cells improves bacteria/electrode interaction and enhances extracellular electron transfer.

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Live Cell Based Bio-Photoelectrochemical Cells for Clean Solar Energy Conversion

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Photosynthetic organisms are attractive starting materials for solar energy conversion (SEC). All of these organisms contain all of the components necessary to create a flux of light-driven electrons, that if harvested can be used directly as an electrical current or to produce clean fuels such as hydrogen. Each organism may have morphological and biochemical characteristics that can benefit SEC under certain conditions. The level of sophistication of the cell, the cross section for light absorption, as determined by the amounts and types of light harvesting antenna complexes (LHCs), the ability to thrive in extreme conditions (light, temperature, ionic strength) can all be utilized by designing the proper Bio-PhotoElectro-Chemical cell (BPEC). A benefit of using intact, live cells is that they have the benefit of evolutionarily optimized LHCs for improved energy absorption and transfer, and that cells have the capability to operate internal repair systems leading to essentially endless activity. However, how these intact systems can be functionally connected to the BPEC depends on the morphology, permeability and flux of electron carriers. We will show here that we can obtain significant electrical current using a variety of live photosynthetic organisms, each with benefits and drawbacks. Cyanobacteria[1,2] and microalgae[3] are applied directly to the BPEC anode, providing continuous current densities in the 10's of □A/cm^2. Use of photosynthetic tissues from macroalgae (seaweeds)[4] or higher plants[5] require tight association with metallic anodes (stainless steel, aluminum, etc.) leading to current densities of >50mA/cm^2, with high ionic strength electrolyte solutions supporting these increased current densities. Succulants can serve as their own BPEC[6]. In all cases, the major electron carrier is NADPH which is released by the cells and tissues into the electrolyte.

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Enhancing microbial biophotoanodes efficiency with functionalized gold nanostructures

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To achieve a more sustainable and ecologically friendly future, a number of appealing technologies have been proposed to meet the rising need for sustainable electricity generation. In this context, biophotovoltaics (BPVs) represent attractive technologies for cost-effective and sustainable electricity generation. These biohybrid electrochemical systems can be obtained by implementing intact, metabolically active photosynthetic bacteria with electrodes, utilizing artificial approaches that allow generating bioelectricity using different substrates as electron donors in the presence of light.[1, 2] Despite the many advantages offered by these technologies, their industrial application is currently limited by low stability and efficiency problems.[3] Due to their unique properties and easy processability, nanomaterials can be used in BPVs to overcome some of the inherent limitations of these devices, such as enhancing extracellular electron transfer (EET) and light harvesting efficiency. Physicochemical properties of nanoparticles (NPs), such as optical resonance wavelengths, extinction cross-section, and relative contribution of scattering to the extinction, can be tuned as a function of composition, size, and shape. Metal NPs, combined with living microorganisms, can be exploited as light absorber to improve light harvesting and as transmembrane and outer-membrane "conductive bridges" to boost charge extraction efficiency from the bacterial cell network to electrode surfaces.[4] In this work, gold nanoparticles of different shape obtained by colloidal synthesis and functionalized with small thiolate molecules, were incubated with *Rhodobacter capsulatus* (*R. capsulatus*) cells in order to develop a microbial biophotoanodes. First, the capability of the bacterial cells to cope with the presence of the metal NPs was confirmed by growth-based viability tests. Following, the bacteria-AuNP interaction was confirmed with TEM micrographs. The effectiveness of the developed biophotoanode in generating electric current was investigated by cyclovoltammetry studies revealing a significant enhancement in both the onset potential and the current density obtained from biophotoanodes with thiolatefunctionalized AuNPs modified *R. capsulatus* cells.

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Light-harvesting processes in green sulfur bacteria *in vivo*

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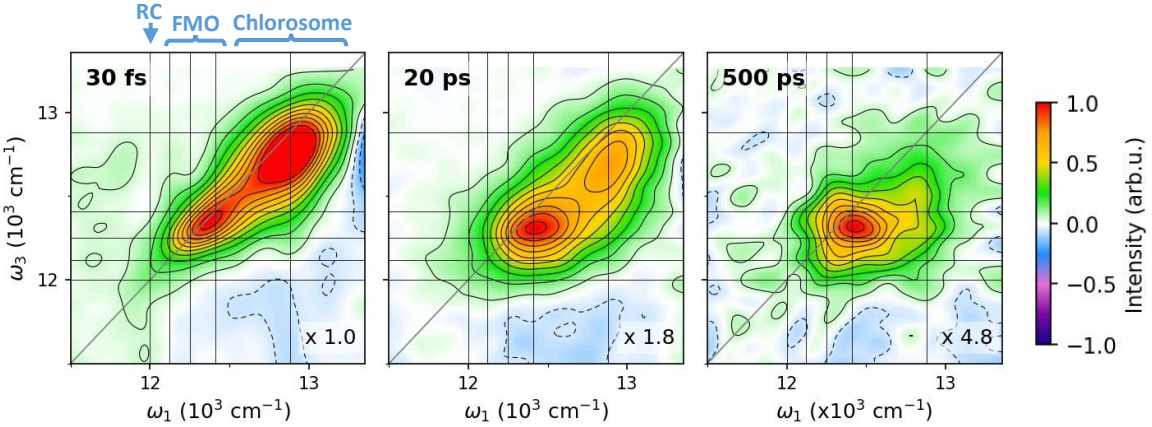
Green sulfur bacteria are remarkable organisms able to perform photosynthesis in environments with extremely low light intensities. Their photosynthetic apparatus, comprising a chlorosome, FennaMatthiews-Olson complexes and reaction centers, has been extensively studied. The structures of these components have now mostly been resolved[1], while spectroscopic studies investigated their functions in light capture, excitation energy transport, and charge separation[2]. However, a large majority of these studies have focused on isolated complexes, preventing obtaining a global picture of energy transfer through the entire apparatus, especially at physiological temperatures. Using two-dimensional electronic spectroscopy (2DES), we characterized energy transfer processes in whole cells of *Chlorobaculum tepidum* at room temperature, drawing on a previous study on the same species at cryogenic temperature[3]. We obtain new insights on the functional connectivity of the complexes in the intact photosynthetic unit at physiological temperature.



Room temperature 2DES spectra of whole cells of the green sulfur bacterium Chlorobaculum tepidum *shown at different population times.*

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Electric polarization effect on photosynthetic efficiency, non-photochemical quenching and photovoltaic performance of intact Limnospira indica based photoelectrodes

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Because of their excellent photosynthetic capability, directly and efficiently applicable in the generation of O_2, the utilization of CO_2, the production of food and chemicals, and the photoelectrochemical production of fuels, cyanobacteria are being deployed by space agencies to establish life support systems in future space stations, planetary stations, and long-haul space missions [1]. In the harsh conditions of Space, the use of live cyanobacteria is preferable to the use of cellular components since cells can cope with and adapt to stress, including ionizing radiation, thus allowing for long-term autonomous operation.

Protein-pigment complexes located in intracellular structures (the thylakoid membranes) capture and transform photonic energies (in the wave length range of 400-700 nm) leading to the liberation of electrons from water into an intricate electron flow which can be either utilized for photosynthesis or driven to external circuit of photovoltaic or photoelectrochemical cell. Excess energy is also dissipated as a heat. The productivity of photosynthesis can be estimated by a combination of electrochemical and spectroscopic methods. Pulse-Amplitude-Modulation (PAM) fluorometry in conjunction with the saturation pulse method has been successful employed for studying induction and quenching of chlorophyll fluorescence in plant physiological studies [2]. Here we have used PAM to study cyanobacterial photosynthetic performance under different polarization conditions. We have found that negatively polarized bioelectrodes based on intact *Limnospira* demonstrate higher photosynthetic light utilization efficiency but low photocurrent. Positively polarized photoelectrodes show less photosynthetic activity but high photocurrent and 'negative heat dissipation' values measured by PAM evidencing that heat dissipation decreases to favor photocurrent in external cirquit.

This work was supported by Research Foundation Flanders (FWO-Flanders) and Swiss National Scientific Foundation under the Senior Lead Agency Project G0D4920N/189455.

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Diatom Microalgae: Overlooked Candidates for the Construction of Robust Biophotovoltaic Devices

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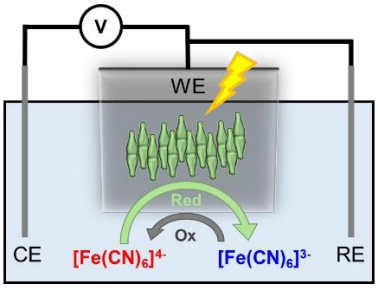
Biophotovoltaic devices are a promising source of renewable energy, given the can generate electrical current from the photosynthetic activity of microalgae, cyanobacteria, or their isolated proteins. Different strategies such as direct electron transfer or, more commonly, the use of soluble electrochemical mediators allow to directly harvest electrons from photosynthetic organisms' cells and feed them to a closed circuit to generate current. Green microalgae have been extensively studied for the extraction of photocurrent, yielding promising outputs, mainly thanks to their easy culture and fast growth.[1] Alternatively, only a few examples are present in literature about the use of Diatom Microalgae for photocurrent extraction.[2] Besides providing significant photocurrent intensities, diatom microalgae exhibit particular resistance to different kind of stressors such as desiccation, temperature and mechanical pressure, which comes as an advantage when it comes to processing them into a living bioanode.[3] Their sensibility to different contaminants make them good candidates for the development of electrochemical biosensing devices.[4] Moreover, diatoms hold the unique characteristic of bearing nanostructured biosilica microscopic shells (frustule), that can be functionalized with active molecules or polymers opens the door to enhancing electron flow, through conductive polymers,[5] or light absorption modulation, for example via introduction of external antennae.[6] This work aims to study the photocurrent extraction from different species of diatom microalgae deposited onto ITO used as working electrode in a three-electrode cell, evaluate their interaction with the electrode and mediator, and their resistance to the electrochemical process itself, particularly in contrast to that of a model green algae. Cyclovoltammetry and chronoamperometry measurements have been performed; both optical and fluorescence microscopies have been used to study the microalgae's state under the different conditions studied. Up to this point, we have standardized a method that allows to produce bioanodes resistant to desiccation, from several different species of diatoms, that provide significant levels of power output.



Three-electrode system for photocurrent extraction from diatom bioanode

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Parallel symposia

Tuesday August 29

Morning

Keratinocytic skin cancers

Invited speakers:

IL33 Franz Trautinger (St. Pölten, Austria)

Keratinocytic skin cancers: clinical presentation, epidemiology, and management

IL34 Fritz Aberger (Salzburg, Austria)

Hedgehog signaling in basal cell carcinoma - Challenges and next generation therapeutics

IL35 Thomas Haarmann-Stemmann (Düsseldorf, Germany)

Role of the aryl hydrocarbon receptor in the pathogenesis of cutaneous squamous cell carcinoma

IL36 Yolanda Gilaberte (Zaragoza, Spain)

Sunscreens in the prevention of keratinocytic skin cancers

Oral communications:

OC35 Jonatan Riber Granborg: The accumulated UVR dose received on a sun vacation is directly reflected in the amount of cyclobutane thymine dimers in urine

OC36 Catharina M. Lerche: Characterization of keratinocyte cancer by mass spectrometry imaging

OC37 Susana Puig: Evaluation of the biological effect in DNA damage repair of a high broad spectrum sunscreen with nicotinamide and panthenol, using 3D Line-field optical coherence tomography

OC38 Katharina Rolfes: UVB radiation-induced skin carcinogenesis and the impact of low dose UVA irradiation

Keratinocytic skin cancers: clinical presentation, epidemiology, and management

Franz Trautinger[1,2]

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Keratinocytic skin cancers (KC) encompass a group of malignancies derived from epidermal keratinocytes, including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). KC are the most prevalent malignancies worldwide, and their incidence has been steadily rising in recent decades. Occupational and recreational sun exposure remains the primary etiological factor, with fair-skinned individuals being particularly susceptible. Other risk factors are advanced age, male gender, immunosuppression, and genetic predisposition. Driving pathomechanisms include inactivation of p53 in SCC and activation of hedgehog signalling in BCC.

BCC typically present as slow-growing translucent nodules or as non-healing, painless ulcers with raised edges. SCC manifest as hyperkeratotic plaques or nodules and are often preceded by actinic keratoses, their typical precursor. SCC almost exclusively occur in chronically sun exposed areas together with clinical signs of “photoaging”, whereas the co-localization of BCC and chronic photodamage is often less apparent. SCC rarely and BCC almost never metastasize.

Treatment of KC is guided by tumour characteristics, patient factors, and extent of the disease. Occurring towards the end of life, treatment decisions must balance immediate treatment-associated versus longterm disease-associated risks. Surgical excision is the standard of care with high cure rates. Superficial lesions can be successfully treated with cryotherapy, topical agents, and photodynamic therapy. KC are highly immunogenic and in advanced tumours immunotherapy with anti-PD-1 antibodies is successfully used. Targeted therapy with inhibitors of the hedgehog pathway is highly effective in advanced BCC.

Because the population at risk is well defined, sun-protection is effective in primary and secondary prevention, and early recognition and treatment can prevent advanced disease, KC are a perfect model for prevention programs.

In conclusion, KC pose a significant health burden, necessitating an understanding of their pathogenesis, epidemiology, clinical presentation, treatment options, and prevention.

Hedgehog signaling in basal cell carcinoma - Challenges and next generation therapeutics

G. Stockmaier[1], S. Varkhande[1], P. W. Krenn[1], D. Hieu-Hoa[1], D. P. Elmer[1], J. Horejs-Hoeck[1], N. Fortelny[1], I. K. Gratz[1] and Fritz Aberger[1]

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Basal cell carcinoma (BCC) is the most common form of skin cancer, with UV exposure being the major environmental risk factor. The disease is caused by mutational activation of the Hedgehog (HH)/GLI signaling pathway, leading to uncontrolled cell proliferation and tumor formation in the absence of an effective anti-tumor response despite a remarkably high mutational burden. Therefore, we aimed to elucidate the underlying reasons for HH/GLI-driven immune evasion in order to improve current treatments and to identify novel therapeutic approaches beyond the use of clinically approved HH/GLI pathway inhibitors, which often lead to the development of resistance and cause severe adverse effects.

We will discuss the challenges of new innovative agents for basal cell carcinoma patients, present recent results from our studies of novel HH/GLI inhibitors, and also provide an outlook on the development of new rational combination therapies aimed at effective inhibition of HH/GLI signaling and reactivation of the anti-tumor immune response. We will also present novel spatial biology technologies and the establishment of humanized 3D and in vivo skin models for dermato-immuno-oncology questions related to HH/GLI-mediated skin carcinogenesis.

Role of the aryl hydrocarbon receptor in the pathogenesis of cutaneous squamous cell carcinoma

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Cutaneous squamous cell carcinoma (SCC) is one the most frequent malignancies in humans. The major risk factor for the development of SCC of the general population is a chronic exposure to ultraviolet (UV) radiation, in particular its UVB part (280 nm – 315 nm). In occupational settings, exposure to certain chemicals, in particular combustion-derived polycyclic aromatic hydrocarbons (PAH), is considered as another important risk factor for the disease. At least to some extent, both UVB radiation and PAHs unleash their carcinogenic potential by activating the aryl hydrocarbon receptor (AHR). The AHR is a ligand-dependent transcription factor expressed in nearly all cutaneous cell-types investigated so far. It maintains skin integrity by regulating xenobiotic metabolism, epidermal barrier formation, pigmentation and other critical processes. However, in response to chronic exposure to environmental stressors, a sustained stimulation of AHR and downstream signaling pathways critically contributes to the development of SCC in mice. In this presentation, I will focus on the central role the cell-cycle inhibitor and tumor suppressor protein $p27^{KIP1}$ is playing in the oncogenic outcome of AHR signaling in environmentally-stressed skin. In fact, a ligand-dependent activation of AHR triggers the cytosolic mislocalization and subsequent proteasomal degradation of $p27^{KIP1}$. This in turn dampens cellular defense mechanisms, such as DNA repair and apoptosis, and thus facilitates tumorigenesis. Our data also indicate that an exposure of epidermal keratinocytes to AHR-activating chemicals (ligands) enhances the genotoxicity of subsequently applied UVB radiation by modulating the nucleotide excision repair of cyclobutane pyrimidine dimers. Hence, a transient inhibition of cutaneous AHR signaling might be a suitable approach to prevent the development of environmentally-induced keratinocyte-derived SCC.

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Sunscreens in the prevention of keratinocytic skin cancers

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Excessive sun exposure is the main cause of keratinocytic skin cancer. Photoprotection in general, and sunscreens in particular, seem to play a crucial role on its prevention. However, there are not many clinical trials that demonstrate the preventive role of sunscreens, and the results of the systematic reviews and metaanalysis are unconclusive. Sunscreens have limitations, especially determined by the way that the population use it. In addition, their composition has changed along the years, increasing their spectrum of protection, not only to the UVB but also to the UVA radiation. Moreover, the addition of other active ingredients to sunscreens, such as antioxidants and especially DNA repair molecules could help to enhance their preventive action. All these aspects, analyzing the existing evidences and also the future trends will be reviewed.

The accumulated UVR dose received on a sun vacation is directly reflected in the amount of cyclobutane thymine dimers in urine

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Exposure to ultraviolet radiation (UVR) is the main carcinogen in skin cancer development. UVR exposure cause DNA damage in the form of cyclobutane pyrimidine dimers (CPDs). These can be used as a risk indicator for developing skin cancer. The formed CPDs are excised from the DNA by nucleotide excision repair (NER) and are excreted with the urine. The most frequent of the CPDs is the T<>T thymidine dimer (Mouret et al. 2006). A new ultra-performance liquid chromatography tandem mass spectrometry method was recently developed for detecting the T<>T thymidine dimers in urine (Lerche et al. 2022).

In this study, this new method was for the first time applied to real life data. Healthy volunteers (n=22) exposed to an abrupt increase in UVR during a winter sun vacation were equipped with a personal electronic UVR dosimeter (Petersen et al. 2014) to measure their UVR exposure during the holiday. Morning urine was collected from the participants before the holiday and on three subsequent days after returning home. The UVR exposure adjusted for exposed skin was shown to be directly correlated to the urine T<>T thymidine dimers after the holiday. The availability of this relatively simple analytical method for measuring DNA damage in urine has great potential for the evaluation of systemic photoprevention and photocarcinogens in the future.

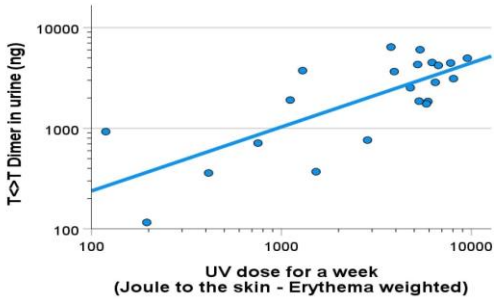

Figure 1: Association between amount of T<>T dimers (ng) in urine and UV dose (Joules to exposed skin - erythema weighted) the last 7 days of vacation. Power model ($P<0.001$, $R^2=0.57$). The cumulative amount of T<>T dimers is calculated from the measured concentration in the morning urine from the first 3 days after vacation.

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Characterization of keratinocyte cancer by mass spectrometry imaging

Lauritz F. Brorsen[1, 2, 3], Fernanda E. Pinto[1], Katja M. S. Bendtsen[4], Merete Haedersdal[1], James McKenzie[3], Zoltan Takats[3], Christian Janfelt[2], Catharina M. Lerche[1,2]

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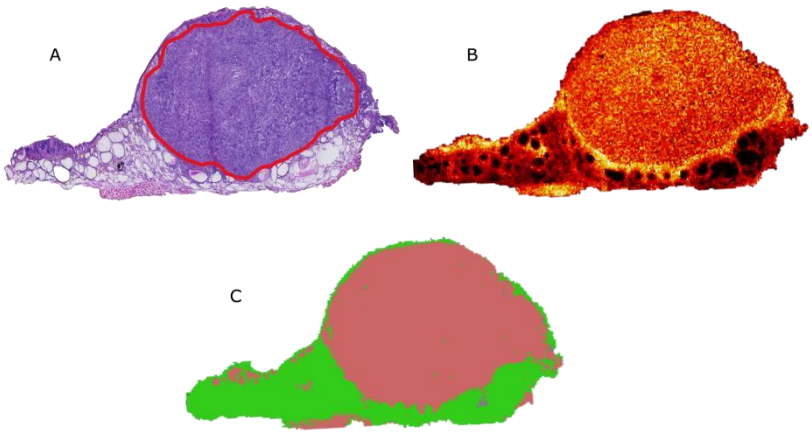
Mass spectrometry imaging (MSI) is an emerging analytical technique that allows visualization and identification of exogenous and endogenous compounds. It combines the high sensitivity and specificity of mass spectrometry with spatial information to create detailed maps of the distribution of molecules within a sample. The aim for this study was to explore the potential of MSI to distinguish cancerous and non-cancerous tissue in murine squamous cell carcinoma (SCC) sections. Matrix-assisted laser desorption-ionization MSI was used to generate datasets of SCCs (n=40). Relevant regions of interest were extracted from the MSI datasets and labelled in accordance with histopathology (e.g. SCC, nontumor tissue, background). A machine learning model (logistic regression) was trained based on the extracted regions. Cross validation indicated an accuracy of < 95% in the MSI datasets. Predictions were compared with pathology. The altered lipid metabolism of the cancerous tissue provides many of the distinguishing features enabling the prediction. These results pave the way for implementation of ambient mass spectrometry techniques, such as laser rapid evaporative ionisation mass spectrometry (LA-REIMS), in clinical oncology for diagnostic and surgical purposes.



A: *H&E staining with annotation of a SCC (red) from a mouse. B: MSI representation of tissue by single ion mapping (mz = 885.55). C: Tumor prediction by a machine learning model trained on MSI data (red = Tumor)*

Evaluation of the biological effect in DNA damage repair of a high broad spectrum sunscreen with nicotinamide and panthenol, using 3D Line-field optical coherence tomography

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Background:

Nicotinamide is the precursor of NAD (nicotinamide adenine dinucleotide), an essential coenzyme in the production of ATP (adenosine thriphosphate), the main source of cellular energy. Previous studies in mice reveal that oral consumption or topical application of nicotinamide prevents immunosuppression and reduces the number of tumors induced by UV radiation [1]. In humans, topical application of 5% nicotinamide prevents immunosuppression caused by solar UV radiation, but not burns [2]. Furthermore, oral nicotinamide reduces the rate of diagnosis of new non-melanoma skin cancers and actinic keratoses in high-risk patients [3]. It has been suggested that one of the mechanisms by which nicotinamide may protect against photodamage is by increasing ATP production which enhances DNA repair [4]. Additionally, nicotinamide acts as a PARP1 inhibitor. Extensive DNA damage leads to overactivation of PARP1 which can lead to NAD depletion. Thus, cells are unable to enter apoptosis, since the process requires a large amount of energy [5].

Hypothesis:

Using a sunscreen with a high broad spectrum UVB-UVA, containing Nicotinamide and Panthenol, can help reverse chronic sun damage to the DNA of skin cells and therefore improve the morphological structure of the skin and keratinocyte nuclei.

Objectives:

To determine the biological effect of a high broad spectrum UVB-UVA sunscreen containing Nicotinamide and Panthenol with the analysis in vivo of the treated skin with LC-OCT.

Methods and study design:

Prospective unicentric study, with a single group of individuals. We included 19 patients with actinic keratosis (AK) who were between 50 and 70 years old. We selected four lesions of each patient in the scalp: 2 AK and 2 subclinic actinic keratoses (SAK). In the screening visit we performed LC-OCT imaging of the 4 lesions and a biopsy of one AK and one SAK. After 8 weeks of applying the product twice a day, we repeated the LC-OCT imaging technique of the two remaining lesions and afterwards we performed a biopsy. Then, we compared the images of AK vs SAK, AK before treatment vs after treatment and SAK before and after treatment. Besides, we performed a keratinocyte analysis (atypia score and keratinocyte metrics).

Results:

We observed that the epidermis and the stratum corneum is significantly thicker for the AK than for the SAK (+51% and +139% respectively). The dermoepidermal junction (DEJ) is more undulated for the AK than for the SAK. Regarding keratynocites analysis, the average number of layers is higher for the AK than for the SAK (+24%) and the atypia score is significantly higher for the AK than for the SAK (+36%). Moreover, the AK keratinocyte nuclei have a larger volume (+9%) with more variability (+19%) and their cytoplasm is larger (+23%). We also observed that the epidermis is significantly thinner in the AK after the treatment than before the treatment (-13%). Regarding the keratinocyte analysis, we found that the mean volume of the SAK nuclei and the average number of layers of the SAK slightly decreases (-8.8% and -7,2% respectively).

Conclusions:

We can conclude that a high broad spectrum UVB-UVA sunscreen containing Nicotinamide and Panthenol might produce a positive biological effect in the structure of the epidermis of AK and the keratinocyte nuclei of SAK. Nevertheless, we did not appreciate statistically significant differences in AK keratynocite analysis and SAK epidermis before and after the treatment. This might be due to a low number of patients involved in this study. Therefore, more studies including more patients should be performed.

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UVB radiation-induced skin carcinogenesis and the impact of low dose UVA irradiation

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Non-melanoma skin cancer including basal cell cell carcinoma and squamous cell carcinoma (SCC) is the most frequent malignancy in humans. Cutaneous SCC develop primarily on sun exposed areas of the body and, in fact, exposure to ultraviolet (UV) radiation is the major cause for SCC. Energy-rich UVB radiation is mainly absorbed by macromolecular structures of epidermal cells in particular DNA of keratinocytes. Accordingly, UVB radiation induced DNA damage needs to be fixed by the DNA repair machinery or cleared through apoptotic demise to prevent mutagenesis. In contrast, UVA radiation penetrates deeper into the skin, reaching dermal fibroblasts and may result in the generation of reactive oxygen species and an associated oxidative DNA damage [1]. Given the fact, that numerousness studies were conducted by exposing test models with either UVA or UVB radiation alone or by sequential irradiation studies, we wondered whether a chronically-applied and biologically relevant noncarcinogenic dose of UVA radiation will affect UVB-induced skin carcinogenesis in SKH-1 hairless mice [2]. Interestingly, preliminary data have shown that in human keratinocytes, simultaneous exposure to low dose UVA radiation reduces UVB-induced DNA double-strand break formation and apoptosis. Hence, we wondered whether a reduced rate of keratinocyte apoptosis after simultaneous UVA and UVB radiation facilitates skin photocarciongenesis. As expected, SKH-1 mice exposed to a low dose UVA radiation as well as the control animals did not develop any SCCs. In contrast, chronic UVB irradiation resulted in an average skin tumour formation of 6 SCCs/mouse. Remarkably, a simultaneous irradiation with UVB and the non-carcinogenic dose of UVA radiation almost doubled the tumour development, as evidenced by a tumour burden of 11 SCCs/mouse. Overall, our data point to the importance of keratinocyte apoptosis for the pathogenesis of skin cancer and highlight the urgent need for further mechanistic research to improve photoprotective measures for a healthy skin.

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Communications in phototherapy and PDT

Oral communications:

OC39 Agnes Marco: Cyclodextrin polymers as carriers of three orthogonal therapeutic agents for the innovative combination of chemo- and phototherapies in hypoxic conditions

OC40 Saulius Bagdonas: Differences in photoinduced transformations of tetrapyrrolic compounds in the presence of L-ascorbate: influence of medium pH and serum albumin

OC41 Catalina Cortes: New Phenalenone like Sensitizers: Expand their use throughout the Blue-Shifted absorption

OC42 Veronika Huntosova: Nanoporous particles for targeted photodynamic therapy of cancer

OC43 Sofia Leo: The radiotherapeutic effects of photosensitizers: An overview of the physical, chemical, and biological mechanisms of radiodynamic therapy

OC44 Hilda Mercado-Uribe: Photoinactivation of E. coli enhanced by oxygen nanostructures.

OC45 Karolina Urbanowicz: Cationic BODIPY complexes based on organoboron scaffolds for microorganisms photoinactivation

OC46 Aleksandra Rapacka-Zdonczyk: Antimicrobial blue light-protective genes in Escherichia coli

OV47 Bianka Siewert: The Photoantimicrobial Power of Polyhydroxyanthraquinones

OC48 Matthew Tung: BODIPY Photosensitizers Modified with Chalcogenophenes

OC49 Li Zhong: 5-Aminolevulinic acid photodynamic therapy activates autophagy by reactive oxygen species to kill Mycobacterium abscessus

OC50 Natalia Burzyńska: Can Staphylococcus aureus develop tolerance to cyclic treatments of aPDI with novel gallium porphyrin derivative?

Cyclodextrin polymers as carriers of three orthogonal therapeutic agents for the innovative combination of chemo- and phototherapies in hypoxic conditions

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Solid cancer cells are often characterized by a low concentration of molecular oxygen (O_2) in the tissues, a condition named hypoxia which activates resistance mechanisms to standard chemotherapy and impairs photodynamic therapy (PDT) relying on the conversion of O_2 into reactive oxygen species (ROS). The H2020 project HypoCyclo aims to develop a nanocarrier combining three therapeutic agents in one platform and overcoming the limitations induced by hypoxic conditions. A biocompatible cyclodextrin polymer forming nanoparticles in water[1] will be loaded with i) a taxane, protecting it and enabling the *in situ* release of the drug; ii) a photosensitizer (PS) allowing PDT; iii) a newly synthesized oxygen releasing agent (ORA) for the supply of O_2 either in its triplet state to feed the PS or as singlet oxygen ($^1O_2$), the most effective ROS. Anthracene and naphthalene endoperoxides have been selected as ORAs and their photocatalyzed synthesis from aromatic substrates has been achieved in the presence of a PS in homogeneous aqueous environment thanks to the use of the cyclodextrin polymer as inert reaction matrix.[2] Co-encapsulation of three agents in the carrier has been achieved in dosage-consistent amounts as confirmed by UV-Vis and emission spectroscopies. Further, the ability of the PS to generate $^1O_2$ and the tendency of the ORA to release O_2 upon thermolysis were unaltered upon complexation in the polymer loading the three components. These preliminary results forecast the use of this polymer as interesting, scalable vessel for the production, carry and delivery of a combination of therapeutic agents.

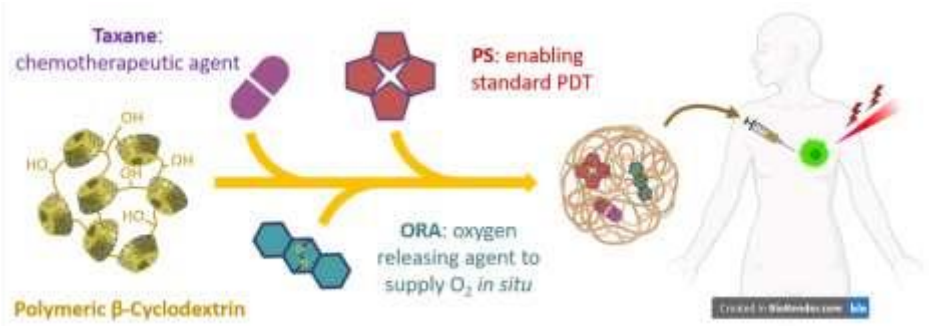

Figure 1. Cartoon representing the strategy for a novel TNBC-targeting combinatorial therapy.

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Differences in photoinduced transformations of tetrapyrrolic compounds in the presence of Lascorbate: influence of medium pH and serum albumin.

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The effectiveness of the photosensitized therapy can be improved by combining it with other methods to increase oxidative stress in tumor tissues, and an L-ascorbic acid (AscA) may serve as a potential candidate [1,2]. Photooxidative reactions initiated by tetrapyrrolic compounds serve not only for biomedical needs but are also applicable in biosensors and green energy production.

Phototransformations of three photosensitizers (PS), hematoporphyrin derivative [3], meso(tetrasulphophenyl)-porphyrin ($TPPS_4$) and aluminum phthalocyanine tetrasulfonate ($AlPcS_4$), were monitored by means of absorption and fluorescence spectroscopy to reveal the effects of AscA on oxygen-dependent photoreactions in aqueous model solutions of different pH as well as in the presence of a bovine serum albumin (BSA). Additional data on the role of the ascorbate radical in photoreactions, as well as on the mutual activity in samples containing BSA, including participation in Type I reactions involving radicals of PS, were obtained by measuring electron paramagnetic resonance (EPR) spectra on samples poured into capillaries of carefully selected diameter and placed inside the Elexsys-E580 spectrometer (Bruker) both in the dark and under illumination with a laser beam through the fiber tip using various wavelengths and intensities.

The strong interaction between BSA and PS was determined as a main factor in the observed photoreactions, not only boosting the photooxidation and photoreduction pathways, but also leading to the enhanced photoactivity in combination with AscA, especially, in acidic medium. The role of individual structural differences in PS, which affect the observed general patterns of their interaction with AscA, will be discussed on the basis of the analyzed spectroscopic data and computer-assisted structural modelling.

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New Phenalenone like Sensitizers: Expand their use throughout the Blue-Shifted absorption

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Phenalenone (PN) is an aromatic polycyclic ketone well known as a type-II photosensitizer, producing singlet molecular oxygen ($^1O_2$) with high quantum yield (Φ_Δ) −close to unity an almost independent on solvent− under irradiation with UV light. [1] Consequently, PN is a good base structure for the design of new sensitizers for the advancement of Photodynamic Therapy (PDT), among other applications. Knowing that increasing π−electron delocalization, a bathochromic shift of the electronic absorption spectra is promoted, substituted PN derivatives should generate a new family of compounds that maintain the photosensitizing capability but using blue light as irradiation source.

We have synthetized, characterized chemically and photo-physically three new sensitizers 1, 2 and 3 (Figure 1) substituted at position 2 and 3, with oxazole, phenyloxazole and benzo-oxazole respectively. They show a lowest-energy absorption band between 400 and 420 nm −nearly independent of solvent polarity−, possess high singlet oxygen generation quantum yields, Φ_Δ, ranging between 0.5 to 1 on different solvents and they show a great photostability after several hours of irradiation.

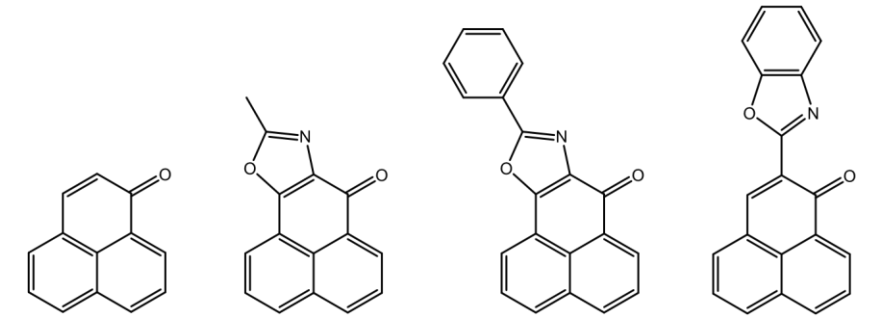

Figure 1: Sensitizers PN, 1, 2 and 3.

Considering that PN presents an electronic absorption maximum at 360 nm approximately, functionalization of heterocycle to PN scaffold was effective to achieve a bathochromic shift of the absorption band with modest variation in Φ_Δ. Consequently, we report a new family of phenalenonebased photosensitizers that are excitable with blue light. These newly reported compounds could open the way to future applications in PDT with possible utilization in antimicrobial treatment.

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Nanoporous particles for targeted photodynamic therapy of cancer

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Currently, nanoporous silica is used in various fields of nano- and micro-material research. The advantage of nanoporous material is that it can be filled with various hydrophilic and hydrophobic molecules that can be delivered to the target cells and tissues. In our work, we investigated the interaction of nanoporous silica with photodynamically active hypericin. Thy hypericin was encapsulated in the pores of the particles. This formulation was modified to allow active targeting to cancer cells. This modification slightly affected the metabolism of the cancer cells, and the targeted transport of hypericin into the cancer cells increased the efficacy of photodynamic therapy. Due to the fluorescence of hypericin, we were able to study the uptake, redistribution, and subcellular localization of the newly produced particles. The applicability of the transport system *in vivo* was investigated using fluorescence pharmacokinetics on the avian chorioallantoic membrane model. In conclusion, nanoporous silica particles were demonstrated to be an effective cargo for hydrophobic photosensitizers with gradual release.

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The radiotherapeutic effects of photosensitizers: An overview of the physical, chemical, and biological mechanisms of radiodynamic therapy

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Significance: Radiotherapy is widely implemented for the treatment of the head and neck, breast, cervix, prostate, eye, thyroid and also gastroenteropancreatic and neuroendocrine tumors [1]. New technologies have paved the way to safer radiotherapy regimens, such as brachytherapy, stereotactic- and intensity-modulated radiotherapy, but the efficacy still requires improvements. Inorganic nanoparticles are frequently explored as radiosensitizing agents [2][3], but studies have also emerged that report on the radiosensitizing properties of porphyrins and other photosensitizers [4]. This approach is referred to as radiodynamic therapy (RDT), yet the underlying mechanisms remain poorly understood. The aim of our research is to shed light the interaction between these photosensitizers and ionizing radiation.

Approach: We systematically summarized the findings of 54 studies in which radiosensitization/RDT with photosensitizers was reported.

Results: Out of 54 papers on radiosensitization/RDT with photosensitizers, 18 papers reported on ALA or PpIX, 6 papers on verteporfin, 7 papers on photofrin and 23 papers on other photosensitizers. The studies have demonstrated that in presence of photosensitizers, radiotherapy causes elevated ROS production in physicochemical experiments [5], and increased cell death and tumor growth inhibition in biological experiments. This was observed in a broad dose-range for diverse types of radiation (e.g., X-ray dose-range 100eV-100keV). To understand how this happens, we reviewed the fundamental mechanisms of radiotherapy. Photosensitizers are typically excited with visible photons of >1.5 eV, and electrons and photons with similar energies are generated via the Auger effect, pair production, Bremsstrahlung effect and Cherenkov radiation. These effects may thus be responsible for the excitation of porphyrins and other photosensitizers, yet controlled experiments to identify the dominating effects are missing. In biological studies, several photosensitizers were shown to interfere with cell signaling events, which was shown to increase the susceptibility of the cells to radiotherapy.

Conclusions: The interactions between secondary electrons and photons during radiotherapy can catalyze the production of toxic reactive species via porphyrins and increase treatment efficiency. Porphyrins and other photosensitizers are therefore promising radiosensitizing agents.

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Photoinactivation of *E. coli* enhanced by oxygen nanostructures.

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Three components are needed to achieve the photoinactivation of a pathogen: a photosensitizer (PS), light of a suitable wavelength and molecular oxygen in the aqueous medium. When light is absorbed by the PS, reactive oxygen species (ROS) are generated. These ROS are responsible for the damage of the biomolecules in the pathogen. Until now, studies have focussed on improving the action of the PS in order to increase such damage. In this work, attention is addressed on the molecular oxygen in the medium, which generates the most toxic ROS: singlet oxygen produced in the so-called Type II mechanism. We exposed *E. coli* cultures with methylene blue to red light. However, the PBS buffer where the bacteria are suspended, is previously enriched by oxygen obtained with high pressures, going from 8 to 300 ppm. Our findings show an enhancement of approximately 1.5log10 in the photoinactivation effect.

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Cationic BODIPY complexes based on organoboron scaffolds for microorganisms photoinactivation

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Singlet oxygen is a valuable reactive oxygen species that could be applied in the synthesis of complex organic systems and in the utilization of compounds present in municipal waste, including antibiotics.[1] Moreover, within living cells, this non-radical reactive form of oxygen can non-selectively oxidize vital biomolecules like lipids, nucleic acids and proteins, leading to cell death.[2] Due to these properties, singlet oxygen shows potential application in photodynamic therapy for treating cancer cells and dermal diseases, as well as in the photoinactivation of various pathogens such as bacteria, fungi, and viruses.[3]

BODIPY complexes, which are derivatives of 4,4-difluoro-4-boro-3*a*,4*a*-diaza-*s*-indiacene, can be used as photosensitizers in order to effectively generate reactive oxygen species, particularly singlet oxygen. Previous efforts to enhance the ability of BODIPY complexes to generate triplet states have focused on ligand functionalization without considering modification to the boron atom. However, our recent studies have introduced boracyclic scaffolds in BODIPY complexes, which exhibit promising properties as homogeneous catalysts.[5] In these compounds, the boron atom acts as a separator between the donor (boracyclic scaffold) and acceptor (ligand) sites. The incorporation of a *spiro* geometry not only enables the generation of triplet states but also opens up opportunities for wider structural modifications within the ligand and boracyclic parts, such as the addition of hydrophilic functional groups that could improve their water-solubility. Herein, I present a series of cationic, heavy-atom free BODIPY complexes based on five different heteroaromatic scaffolds. Obtained compounds were examined as potential photosensitizers for photoinactivation of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans.* Moreover, the cytotoxicity of synthesized systems in mammalian cells were investigated.

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Antimicrobial blue light-protective genes in *Escherichia coli*

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Antimicrobial blue light (aBL) is an innovative light-based non-antibiotic approach to combat multidrug resistant microorganisms. aBL is considered as safe alternative to thermal approaches and ultraviolet (UV) radiation, with little risk of resistance development. The effectiveness of aBL is believed to be the result of a multi-target mode of action. Nevertheless, it must be admitted that the entire mechanism of action of aBL is not yet fully understood.

The aim of this study was to identify genes and proteins that are substantial for the bacterial response to aBL in *E. coli*. The source for genome-wide testing of mutational effects was the Keio knockout collection, a set of 3 985 single-gene mutants of non-essential genes[1].

After analysis of the entire Keio collection, 64 single-gene mutants that express the aBL hypersensitivity phenotype were distinguished. The performed screening revealed that these genes contribute to a wide range of biological processes, including metabolism, biosynthesis, regulation, DNA repair and stress response. The complementation of the deleted genes with ASKA library[2] restored the wild-type phenotype of aBL sensitivity to hypersensitive mutants.

Current study contributes to a better understanding of the fact that the response to aBL depends on many factors and that it may be difficult to identify the dominant mechanism underlying the sensitivity of microorganisms to photoinactivation.

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The Photoantimicrobial Power of Polyhydroxyanthraquinones

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Polyhydroxyanthraquinones, especially those from fungi, have recently been recognized as an underappreciated source of natural photosensitizers. Since fungi share their ecological niche with other microorganisms, we hypothesized that such anthraquinones act as photoantimicrobials. To test our hypothesis, several anthraquinones were tested against yeasts (e.g., *Candida albicans*, *C. auris*, and *Saccharomyces cerevisiae*) and different bacteria (e.g., *Escherichia coli* and *Staphylococcus aureus*) under different irradiation conditions (λ = 428, 478, or 528 nm, H = 30 J cm^{-2}) and in the dark using a standardized method based on CLSI and EUCAST protocols. A significant photoantimicrobial effect was observed for various monomeric anthraquinones (pMIC < 1 mg mL^{-1}), while dimeric anthraquinones were characterized by a weaker photoantimicrobial effect (e.g., Skyrin $pMIC_{428}$ = 15 µg mL^{-1}). To investigate the structure-activity-relationship of the isolated natural and related synthetic anthraquinones, the photoyield of singlet oxygen production was determined and correlation studies were performed. Furthermore, cellular uptake was investigated by HPLC and fluorescence microscopy and the intracellular production of singlet oxygen in cells explored.

In conclusion, we demonstrate here the remarkable potential of polyhydroxy anthraquinones against gram-positive and gram-negative bacteria as well as some yeasts that cause unpleasant skin infections in humans.

BODIPY Photosensitizers Modified with Chalcogenophenes

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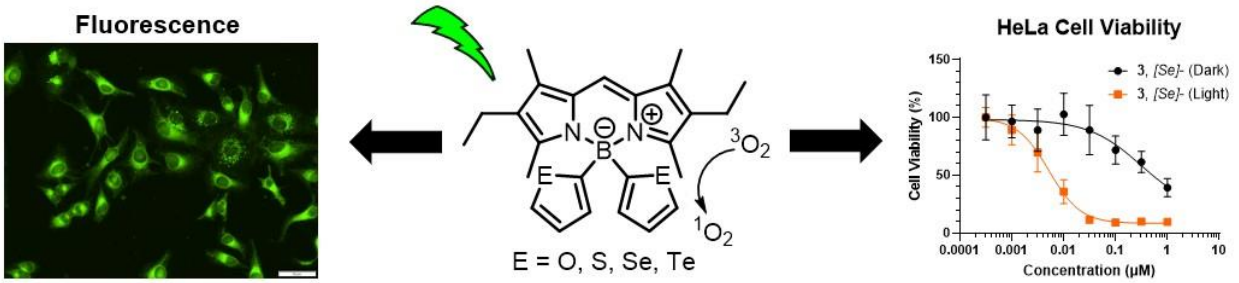
Boron-dipyrromethene dyes (BODIPYs) are a class of fluorophores that have been widely used due to their ease of synthesis, tunability, high fluorescence quantum yields and strong extinction coefficients[1]. They have also been shown to become potent photosensitizers (PS) upon modification with heavy atoms[2], or by introducing donor-acceptor moieties[3]. Although promising, most modifications are done on the dipyrrin core, thus limiting the design of BODIPY-based PSs as the core is commonly used to red-shift the absorbance of the PS, functionalize, and introduce selectivity toward target cells[1]. To overcome this, we have developed a series of BODIPY PSs modified at the boronic position with tellurophene, selenophene, thiophene, or furan rings to explore their capabilities as novel PSs[4]. All compounds exhibited high photostability, improved singlet oxygen generation compared to native BODIPY, and most retained modest fluorescence quantum yields. Cell viability assays were conducted with HeLa cells and nanomolar IC_{50} values were observed when irradiated for five minutes with a 525 nm lamp (15.60mW/cm^2)[4]. Overall, my talk will describe the synthesis and characterization of BODIPYs modified with chalcogenophenes and introduce an original method to convert BODIPYs into potent PSs whilst retaining their tunability.



Fluorescence and photosensitization of chalcogenophene-modified BODIPYs in HeLa cells

Reference

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5-Aminolevulinic acid photodynamic therapy activates autophagy by reactive oxygen species to kill *Mycobacterium abscessus*

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Infections caused by nontuberculous mycobacteria are increasing year by year. Among them, *Mycobacterium abscessus* is the most common nontuberculous mycobacteria, which can cause severe respiratory, skin and mucous membrane infections in humans. In recent years, *Mycobacterium abscessus* Infections increasing year by year, it has become a major emerging pathogen and one of the most antibiotic-resistant nontuberculous mycobacteria, poses a great challenge to clinical treatment. Photodynamic therapy is a novel treatment for diseases that do not develop drug resistance. To investigate the effect of ALA-PDT on *Mycobacterium abscessus* and its molecular mechanism, we conducted a series of *in vitro* and *in vitro* experiments. The results showed that ALA-PDT activated autophagy to kill *Mycobacterium abscessus* through reactive oxygen species (ROS), and it was proved that E1A Binding Protein P300 (EP300) has an important regulatory role in killing *Mycobacterium abscessus* by ALA-PDT. This study provides a new option for the treatment of *Mycobacterium abscessus* infection, and at the same time provides a theoretical basis for the wide application of clinical ALA-PDT.

Can *Staphylococcus aureus* develop tolerance to cyclic treatments of aPDI with novel gallium porphyrin derivative?

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Staphylococcus aureus is one of the primary pathogens that can cause several infectious diseases in both hospital and community settings. Moreover, because of continuously raising of antibiotic resistance, treating of *S. aureus* infections is exceedingly challenging [1][2]. Antimicrobial photodynamic inactivation (aPDI) is a promising alternative approach with proven high bactericidal efficacy with different types of photosensitizers toward various microbes regardless of antibiotic resistance [3]. In contrast to antibiotics, aPDI is considered as a low-risk approach for the development of resistance, but it was reported that tolerance to this treatment may emerge over time [4]. The current study aimed to investigate whether *S. aureus* can develop tolerance to aPDI with novel gallium metalloporphyrin.

Reference *S. aureus* ATCC 25923 was subjected to cyclic treatments of sub-lethal aPDI with Ga-CHP 2-3 as a photosensitizer and a blue light (λ_{max} = 409 nm). Throughout 15 cycles 0.2 µM GaCHP 2-3 and 6.2 J/cm^2 was applied for aPDI and control cultures were passaged through cycles with no treatment. In day 1th,5th,10th and 15th cultures were subjected to 0.2 µM Ga-CHP 2-3 and 0-18.7 J/cm^2 to determine tolerance development and streaked to agar plates with rifampicin to assess mutation rate.

After the 5th cycle of the treatment, the tolerance was observed and persisted at the constant level throughout remaining 10 cycles. Nevertheless, culturing for 5 cycles without aPDI exposure resulted in restored original phenotype. Additionally, the outcomes of rifampicin-resistant mutant selection assay revealed no increased mutation rate in *S. aureus* upon sub-lethal phototreatments.

All this leads to the conclusion that *S. aureus* may develop tolerance to studied phototreatment upon sub-lethal doses but this tolerance is non-inherited and does not remain stable.

Reference

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Light in dentistry

Invited speakers:

IL37 Ellen Bruzell (Oslo, Norway)

Blue light hazard from light curing devices for dental materials: 20 years of monitoring

IL38 Silvia Nuñez (São Paulo, Brazil)

Microbial resistance in oral cavity: challenges and opportunities in antimicrobial photodynamic therapy

IL51 Martha Simões Ribeiro (São Paulo, Brazil)

Light-based technologies in Dentistry: from basis to clinical practice

Blue light hazard from light curing devices for dental materials – 20 years of monitoring

Ellen Bruzell

Nordic Institute of Dental Materials (NIOM), Oslo, Norway

Light curing devices are used in dentistry to polymerise composite resin dental materials containing one or two photoinitiators. Following the outphasing of devices with quartz-halogen and plasma arc lamps emitting broad spectra of UVA and visible radiation in the range ~350-600 nm, today's devices are made with light emitting diode (LED) technology characterised by one or two narrow (30-60 nm) spectral peaks within the range ~390-520 nm. Spectral irradiance (W/m^2) of the LED curing devices, having emission diameters of ~ 0.4-12 mm, has increased several-fold since the products were introduced on the market. Their output in the blue wavelength range overlaps to various extents with the retinal blue light hazard function. ICNIRP guidelines (1) and ISO standards (2, 3) aid in evaluation of the risk of eye damage from exposure to blue light emitting curing devices. Different scenarios have been set up to evaluate the risk from reflected or direct exposure to curing devices from which emission is directed to a material in the patient's oral cavity. Depending on scenario and curing light radiance ($W/m^2/sr$), limit values set for blue light can be exceeded. However, the uncertainty associated with the exposure is large. Nevertheless, the use of appropriate eye protection would greatly reduce any risk to the curing device operator.

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Microbial resistance in oral cavity: challenges and opportunities in antimicrobial photodynamic therapy

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Microbial resistance in the oral cavity is a growing concern, as it can lead to persistent infections that may have consequences in general health with limited treatment options. Antimicrobial photodynamic therapy (aPDT) offers a potential solution to combat this issue. Bacteria in the oral cavity often form biofilms, which are complex communities encased in a protective matrix. Biofilms make bacteria more resistant to antibiotics and antimicrobial agents, including aPDT. The structure of biofilms impedes the penetration of photosensitizers and light, reducing the effectiveness of aPDT. The action of aPDT over the biofilm attached layer may be an advantage to combine therapies. Besides the oral cavity harbors a diverse range of microorganisms and each one may respond differently to aPDT, requiring a tailored approach for effective treatment. Developing protocols that target multiple types of microorganisms while minimizing damage to host tissues is a challenge. Beyond the challenges aPDT offers a nonantibiotic alternative for managing microbial infections in the oral cavity. by targeting multiple microbial species simultaneously, aPDT may help reduce the selection pressure for resistance development. Combining aPDT with other antimicrobial agents or conventional therapies may enhance its effectiveness. For instance, aPDT can be used in conjunction with antibiotics or antifungal agents to improve their microbial killing potential, thus it may overcome resistance mechanisms and provide more comprehensive treatment outcomes using low antimicrobial concentrations which can be important due to the antimicrobial resistance crisis that we face nowadays. Moreover, a combination of antimicrobial treatment with a probiotic approach may provide a personalized treatment tailored to individual patients. In conclusion, antimicrobial photodynamic therapy holds promise in addressing microbial resistance in the oral cavity. Despite the challenges posed by biofilms, microbial diversity, photosensitizer selection, and light penetration, the opportunities for nonantibiotic treatment, synergistic approaches, personalized medicine, and technological advancements offer hope for effective management of oral microbial infections.

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Light-based technologies in Dentistry: from basis to clinical practice

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Nonthermal light-based technologies are becoming more popular as non-invasive and cost-effective therapeutic approaches. These strategies primarily involve photobiomodulation (PBM) and photodynamic therapy (PDT), which use visible or near-infrared (NIR) light to trigger biological effects without significantly warming the body. To improve light penetration into biological tissues in PBM, red or NIR light is typically utilized. The absorption of photons by natural photoacceptors induces photophysical and/or photochemical reactions within cells that drive biological responses such as faster healing of wounds, regulation of inflammatory processes, and pain alleviation, depending on light parameters and target tissue. On the other hand, PDT employs photosensitizing drugs (PSs), which absorb light in the presence of oxygen, to elicit chemical reactions that kill cells through oxidative stress. As with PBM, the success of PDT depends on different factors, such as PS and light parameters, target cells, and oxygen abundance, among others. Our group has been investigating the mechanisms and applications of PBM and antimicrobial PDT (APDT) in dentistry for over 20 years. We demonstrated that PBM can accelerate the orthodontic movement of teeth and promote a faster post-gingivectomy recovery. APDT can be an effective adjunct in endodontics and periodontics and a non-invasive treatment for caries and oral candidiasis. This lecture will focus on basic and applied research for the successful application of light-based technologies in oral care and highlight future directions.

Communications in physical and chemical photobiology

Oral communications:

OC52 Gustavo Cardenas: Influence of the Protonation States on the Absorption and Emission Properties of Rhodopsins

OC53 Zong Jie Cui: Photodynamic Biology: Photodynamic Modulation of Physiologically Important Functional Proteins

OC54 Ariel García Fleitas: Influence of Structural Colour in Molecular Mechanism of Photosynthetic Light Harvesting in Chondrus crispus.

OC55 Krystyna Herasymenko: Ultrafast excited state dynamics of archae-rhodopsin 3 and its mutants

OC56 Matilde Ibba: Photophysics of sunscreen-based photocages containing avobenzone

OC57 Francesca Laneri: Green Synthesis of Near-Infrared Plasmonic Gold Nanostructures by Pomegranate Extract and Their Supramolecular Assembling with Chemo- and Photo-Therapeutics

OC58 Anna Theresa Ott: Sequence-Dependent Interaction between Amotosalen and DNA

OC59 Samim Sardar: Insights into the molecular mechanisms of light harvesting in a minor antenna of plants: CP29 in near-native membrane lipidic environment

OC60 Juan Antonio Soler Orenes: Photophysical properties and in vitro phototoxic effect of adapalene

OC61 Marcel Fuciman: Changes of ICT State Dynamics of 8'-apo-Î²-Carotenal Induced by External Voltage in Electrochemical Cell

ESP2023 Thesis Award

Carla Arnau del Valle: A highly photostable and versatile two-photon fluorescent probe for the detection of intracellular nitric oxide concentrations in macrophages and endothelial cells

Flash presentations (see poster sessions for abstracts):

Cornelia Böhm: Structural Determinants of Bacteriophytochrome Photocycle

Letícia Da Mata Lazinski: Synthesis of hemiindigoids as photoswitchable entities: a spatiotemporal handling of enzyme activity using light

Linda Eijsink: Shining Light on the Mutual Interaction of Photo-responsive Labels and Biomolecules

David T. Griffin: The effects of delivery irradiance on the antibacterial efficacy of 222 nm Far-UVC light

Influence of the Protonation States on the Absorption and Emission Properties of Rhodopsins

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2 Dipartimento di Biotecnologie, Chimica e Farmacia, Università di Siena, via A. Moro 2, I-53100 Siena, Italy

3 Department of Chemistry, Bowling Green State University, Bowling Green, OH 43403, USA

Membrane-bound rhodopsins are a widely spread family of photoresponsive proteins which are involved in a manifold of biological processes spanning from ion-gating, ion-pumping, vision to chromatic adaptation, among others.[1] The functionality of a rhodopsin depends on the retinal chromophore, the photodynamics of which can be tuned by the protein sequence. As a result, the study of the variations in the electronic properties of rhodopsins following certain variations in the protein sequence is of interest not only in the field of photobiology,[2] but also within the frameworks of optogenetics and artificial molecular devices due to the tunability of the properties of the rhodopsin proteins.[3]

In this regard, the Automatic Rhodopsin Modeling (ARM) protocol has recently been developed to simulate photon absorption or emission on both wild type and mutant rhodopsins on a unified comparable model.[4] In this talk, we introduce a previously proposed minimal electrostatic model[5] as an extension of the ARM protocol to account for pH effects by including the relevant protonation microstates that contribute significantly to the overall λ_{max}. As the standard ARM considers a single rhodopsin structure, and thus, a single protonation state, this modification encompasses in an automated fashion less populated protonation microstates that nonetheless have a non-negligible effect on the spectral lineshape and the λ_{max}. Such a mechanism will be illustrated using ArchaeRhodopsin-3, GloeoRhodopsin proteins and mutants.

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Photodynamic Biology – Photodynamic Modulation of Physiologically Important Functional Proteins

Zong Jie Cui [1], Xiao Bing Xie [1], Wei Mei Huang [1], Peng Juan Li [1], Jie Wang [1], Yuan Li [1], Wen Yi Jiang [1], Hong Ning Jiang [1]

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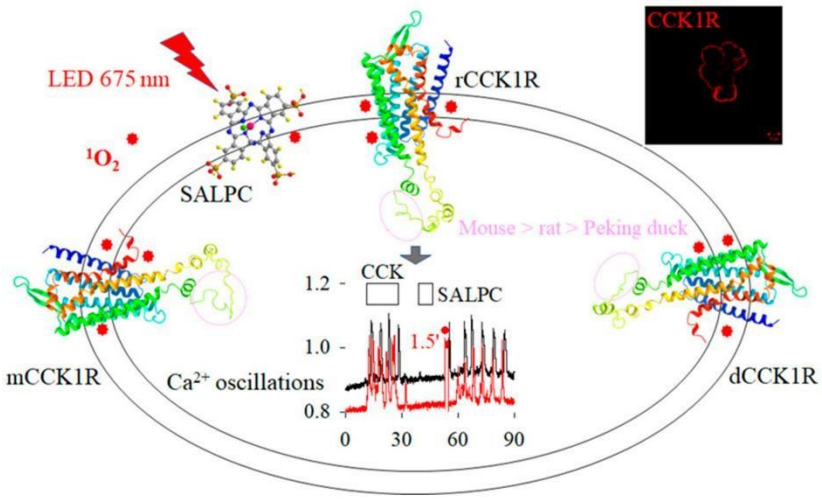
Type II photodynamic action, with the production of excited state delta singlet oxygen (Δ^1O_2), modulates numerous cellular functions including cell secretion, muscle contraction, cell proliferation, cell death and senescence, development, gene transcription and translation, and cellular signaling, all via oxidative modification of specific functional proteins. Photodynamic action on large numbers of functional proteins has been carried out aiming for diagnostic, therapeutic, and investigative purposes. Typical cases of photodynamic modulation of major categories of function proteins will be highlighted in this report, including members of major categories of functional proteins as defined by the International Union of Basic and Clinical Pharmacology (**https://bpspubs.onlinelibrary.wiley.com/toc/14765381/2021/178/S1**): 1/ G protein coupled receptors; 2/ ionic channels; 3/ transporters; 4/ enzymes; 5/ catalytic receptors; 6/ nuclear hormone receptors; 7/ other functional proteins. Particular emphasis will be on photodynamic activation of cholecystokinin receptor type 1, in photodynamic action with both chemical photosensitizer (sulphonated aluminium phthalocyanine, SALPC) and genetically encoded protein photosensitizers (miniSOG and KillerRed).



Photodynamic CCK1R activation is conserved in mammalian and avian pancreatic acini (Wang J, Cui ZJ, 2023)

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Influence of Structural Colour in Molecular Mechanism of Photosynthetic Light Harvesting in *Chondrus crispus*.

Ariel García Fleitas[1,2], Samim Sardar[1], Margot Minju Arnould-Pétré[3], Maria Murace[4], Silvia Vignolini[4], Juliet, Brodie[3] Guglielmo Lanzani[1,2], Cosimo D'Andrea[1,2*]

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Chondrus Crispus, red macroalgae, exhibits photonic structures in the isomorphic state gametophyte and are absent in the tetrasporophyte state of its lifecycle. Here, we have investigated the influence of structural coloration in the molecular mechanism of photosynthesis in the algae using time-resolved photoluminescence (TRPL) studies. We found that the lifetime of the pigments PE, PC and APC increases with an intensification in excitation intensity and the increment saturates toward the higher intensities. This observation can be attributed to the intensity-dependent photoprotection mechanism present in Chondrus crispus. Simulating the role of structural coloration in tetrasporophyte a similarity of the traces after intensity normalization considering the SC, confirms the role of SC in gametophyte in attenuating the photon flux reaching the photosynthetic organisms. These results show that a light management mechanism is present in the external antenna of the red algae associated with longer dynamics where SC on gametophyte tip helps in reducing the number of photons absorbed directly by the Chl and favoring the EET through the external antenna.

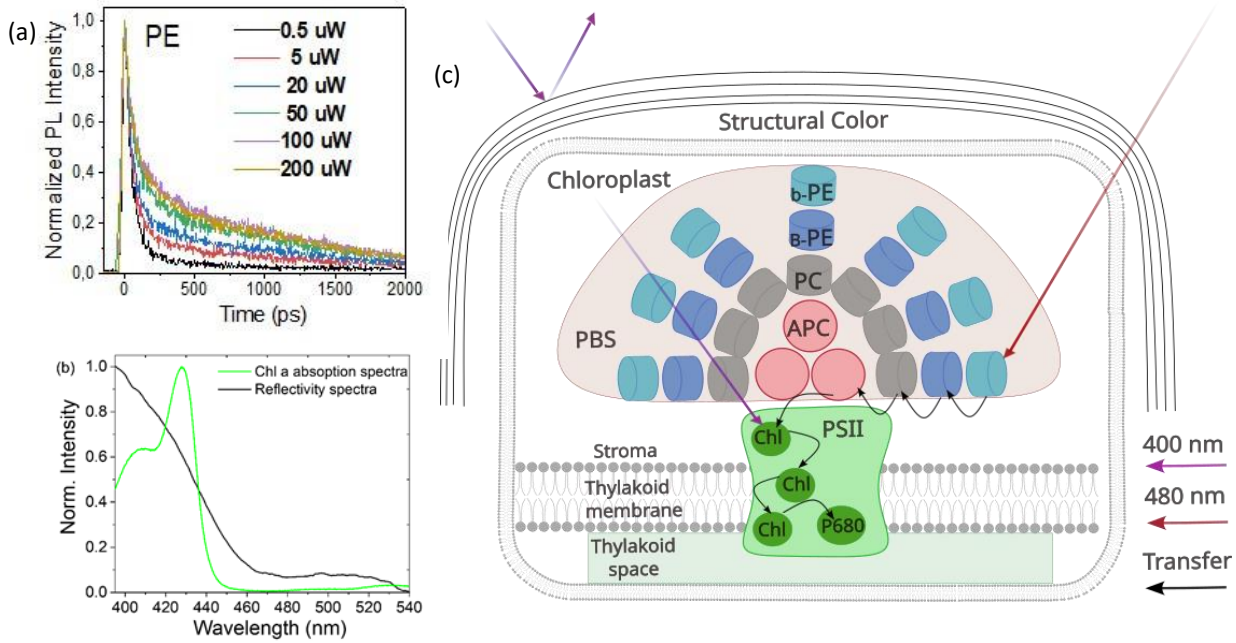

Figure 1:a) Temporal dynamic of the phycoerythrin band of Chondrus Crispus, without structural coloration, b) overlap of the reflectivity of the structural color and the absorption of chlorophyll a; c) diagram explaining the role of structural coloration on the light harvesting mechanism combined with the accessory pigments.

Ultrafast excited state dynamics of archae-rhodopsin 3 and its mutants

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3 PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama, 332-0012, JAPAN

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Archaerhodopsin-3 (AR-3) is a light-driven proton pump found in *Halorubrum sodomense*, which has a photo-cycle similar to that of bacterio-rhodopsin. AR-3 was reported to display a detectable fluorescence, which, when integrated in membranes of live cells, was shown to depend strongly on the transmembrane voltage. AR-3 was then put forward as a possible candidate for optogenetic investigations, i.e. in the form of a genetically encoded voltage indicator [1] to track neuronal electric signals or for neural silencing. Also, multiple mutants then emerged[2], with fluorescence quantum yields (FQY) reaching up to 1.2% which is a 100-fold increase with respect to the wild-type protein (wt). To understand this exceptionally strong effect of the mutations in detail, we studied the fluorescence decay kinetics for wt as a function of pH ≤ 6, since protonation of the counter ion is known to prolong the excited state lifetime of rhodopsins[3]. Other changes in terms of the electrostatic interactions of the protein cavity containing retinal chromophore are induced in the double mutant DETC and the quintuple mutant Arch-5[2].

The excited state kinetics (Fig. 1) are measured with 150 fs time resolution using broadband fluorescence up-conversion and transient absorption set-ups. We find them to be best described by a sum of 3 decaying exponentials, which represent the heterogeneity of protein environment. The average excited state lifetimes reach high values up to *65 ps* (Fig. 1), in good agreement with the measured FQY's[3]. The excited-state lifetime increases for the different AR-3 *logarithmic.* mutants because of a potential barrier in the excited state induced by the interactions with the protein environment[4], but the origin of the three different lifetimes is an open question. The influence of a possible mixture of all-trans and 13-cis isomers on the different excited state lifetimes is currently investigated by controlling the so-called light-adaptation conditions, which assure a dominant all-trans configuration.

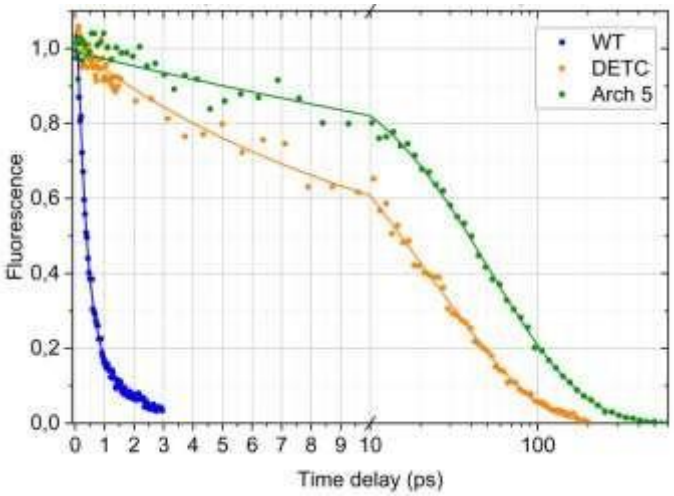

Fig. 1: Fluorescence decays of AR-3, and the two mutants DETC and Arch-5, at pH6. Time axis linear until 10 ps, then

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Photophysics of sunscreen-based photocages containing avobenzone

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Topical drugs are not always innocuous and present drawbacks mainly associated with their interaction with solar radiation. In this context, non-steroidal anti-inflammatory agent (NSAIDs) are one of the most representative examples of photosensitive drugs due to its extensive use in daily life. [1]

Sunscreen-based photocages (ie. a covalently linked pro-drug/pro-filter systems) have been developed to overcome this issue, and to allow controlled and simultaneous photorelease of the masked drug and the solar filter. Previous results using ketoprofen (KP) as NSAID pointed toward the importance of the relative energies of its triplet excited state and that of the masked filter, the avobenzone in its diketone form (AB(K)). [2] To get further evidence that the ^{3}AB(K)* needs to lie at a lower energy than its caged compound, a new dyad made with naproxen (NPX) and the well-established solar filter avobenzone (AB) has been synthesized, studied in EtOH, and compared with AB-KP by means of spectroscopic techniques.s

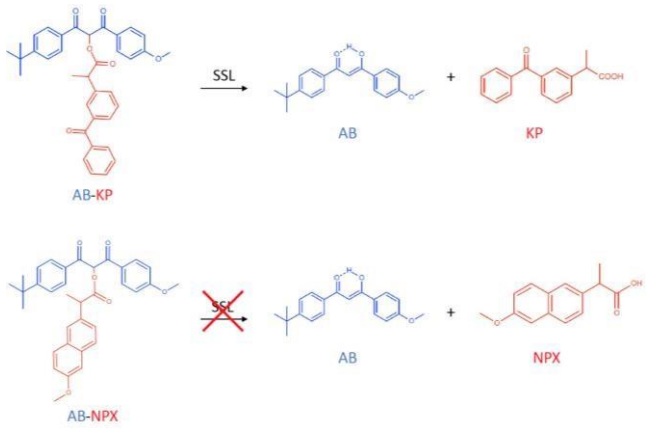

***Figure 1**. Phototriggered release of NSAIDs.*

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Green Synthesis of Near-Infrared Plasmonic Gold Nanostructures by Pomegranate Extract and Their Supramolecular Assembling with Chemo- and Photo-Therapeutics

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Au nanostructures exhibiting a localized surface plasmon resonance in the near-infrared (NIR) spectral window are obtained in a single, green step by pomegranate extract in the presence of a biocompatible β-cyclodextrin branched polymer, without the need of preformed seeds, external reducing and sacrificial agents, and conventional surfactants. The polymeric component makes the Au nanostructures water dispersible water, stable for weeks and permits their supramolecular assembling with the chemotherapeutic sorafenib (SRB) and a Rhodamine- bond nitric oxide (NO) photodonor (RD-NO), chosen as representative for chemo- and photo-therapeutics. Irradiation of the plasmonic Au nanostructures in the therapeutic window with 808 nm laser light results in a good photothermal response, which (i) is not affected by the presence of either the chemo- or the phototherapeutic guests and (ii) does not lead to their photoinduced decomposition. Besides, irradiation of the hybrid Au nanoassembly with the highly biocompatible green light results in the NO release from the RD-NO with efficiency similar to that observed for the free guest. Preliminary biological experiments against Hep-G2 hepatocarcinoma cell line are also reported.

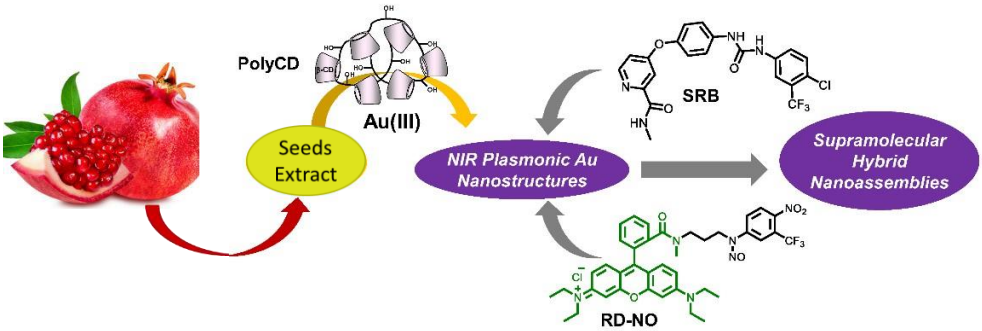

Fig. 1 – Schematic preparation of the NIR plasmonic Au nanostructures and their supramolecular assembling with SRB and RD-NO.

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Sequence-Dependent Interaction between Amotosalen and DNA

Theresa Ott[1], Michelle P. Rademacher[1], Wiebke Haselbach[1], and Peter Gilch

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Pathogens, such as viruses and bacteria, pose a medical risk to the use of blood products. The *Intercept™ Blood System* [1] is an efficient pathogen-inactivation-system to reduce blood product contamination. The synthetic psoralen derivative Amotosalen (AMO, Figure 1, left) reversibly intercalates between base pairs of the target DNA (or RNA). Subsequent photoexcitation by UV light leads to the formation of irreversible bonds between the drug and thymine (or uracil) bases of the DNA (or RNA), causing the inactivation of the pathogens. Earlier work by our group with synthetic DNA containing only GC or only AT base pairs indicates that photo-excited psoralens can be quenched by photo-induced electron transfer (PET) in addition to the desired photo-addition, depending on the base pair environment [2,3]. Here, the DNA sequence complexity was increased with two "mixed" DNA sequences, including all DNA bases (Figure 1, right).

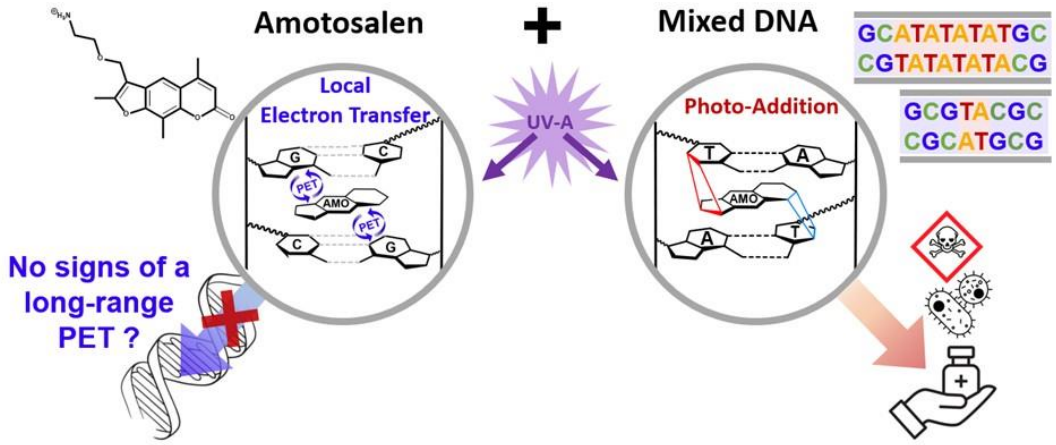

Figure 1: Interaction of Amotosalen (AMO) with "mixed" DNA. Photo-induced electron transfer (PET) with guanine bases is shown on the left. No evidence for a long-range DNA-mediated PET was found. The photo-addition with thymine bases, which is used for pathogen inactivation, is shown on the right.

Steady-state UV-A exposure experiments and time-resolved spectroscopy on the nanosecond time-scale indicate photoproduct formation for both DNA sequences. In addition to the desired photoreactivity, a PET was found in both DNA sequences in the presence of guanine bases. Its driving force and distance dependence can be described by the Marcus theory [4]. The time constants for the PET process range between 15 ps and 20 ps and coincide with those for pure GC DNA. This suggests that the PET in the "mixed" sequences occurs locally in the GC intercalation sites. No evidence of long-range, DNA-mediated PET, as seen in the works by Lewis et al. [5] has yet been found.

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Insights into the molecular mechanisms of light harvesting in a minor antenna of plants: CP29 in near-native membrane lipidic environment

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CP29, a chlorophyll (Chl) *a*/*b*-xanthophyll binding protein, bridges energy transfer between the major LHCII antenna complexes and Photosystem II reaction centres. Until now, the photophysics of CP29 has been studied on the purified protein in detergent solutions, since spectrally overlapping signals affect in vivo measurements. However, the protein in detergent assumes non-native conformations compared to its physiological state in the thylakoid membrane. Here, we report a detailed photophysical study using time-resolved fluorescence and femtosecond transient absorption on CP29 and mutants inserted in discoidal lipid bilayers, known as nanodiscs, which mimic the native membrane environment. CP29 in nanodisc is in a significantly quenched state compared to when it is in detergent. Global analysis suggests an energy transfer from the Chl *a* Q_y to a Car dark state S*, which is generated due to conformational changes in the Car S_1 state. We suggest that the accessibility of the S* state in different local environments plays a key role in determining the quenching of Chl excited states. Our observations shed light on the influence of local environment on the photophysics of CP29, elucidating the importance of a near-native environment when studying the mechanisms underlying the photoinduced dynamics. We have also investigated the role of Zea in the photoprotection mechanism, which exhibit enhanced quenching of Chl emission in the presence of Zea. Finally, the role of individual chromophores in the light regulation processes are investigated on CP29 mutants, affected on pigment binding sites.

References: *J. Chem. Phys.* 156, 205101 (2022).

Photophysical properties and *in vitro* phototoxic effect of adapalene

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Adapalene, a third-generation topical retinoid, is used as a treatment for acne vulgaris. It is currently one of the three topical retinoids approved so far by the Food and Drug Administration (FDA), along with tazarotene and tretinoin.[1] However, a combination of adapalene and sun exposure might result in the appearance of adverse effects. They have been associated with different causes such as skin irritation that can modify the natural skin photoprotection and increase ultraviolet light harmful potential, or a decrease in the thickness of the stratum corneum reducing the natural photobarrier.[2]

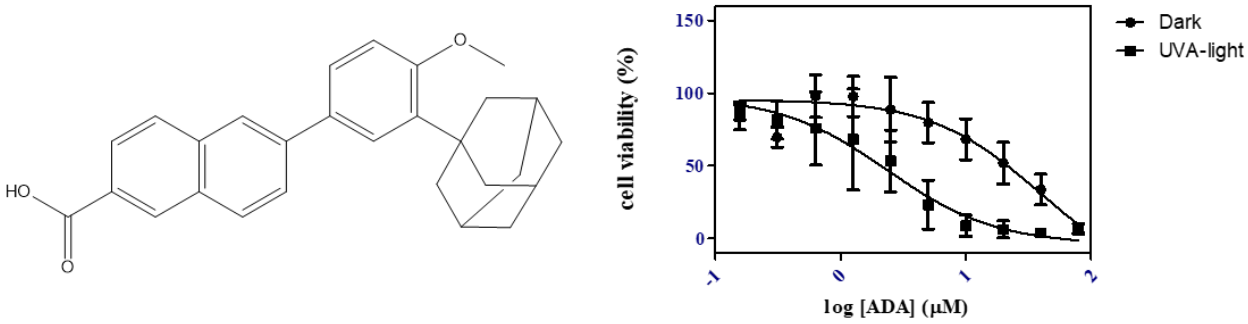

Figure 1. Left, the structure of the adapalene molecule. Right, cell viability at different concentrations of adapalene in the absence of light (circles) and under UVA irradiation (squares).

The aim of this study is to evaluate the phototoxic potential of adapalene. In a first stage, the photophysical properties of the drug were investigated to get more insight into its excited states and their potential to trigger biomolecule damages. For this, experiments were carried out combining fluorescence, phosphorescence (both steady-state and time-resolved emission), and transient absorption at nanosecond-microsecond timescale. Then, adapalene phototoxicity was established *in vitro* using the standard Balb/c 3T3 NRU assay. A high photoirritation factor (PIF) of 16.6 was found, this value is much greater than the threshold of 5 set by the guidelines for phototoxic compounds.[3]

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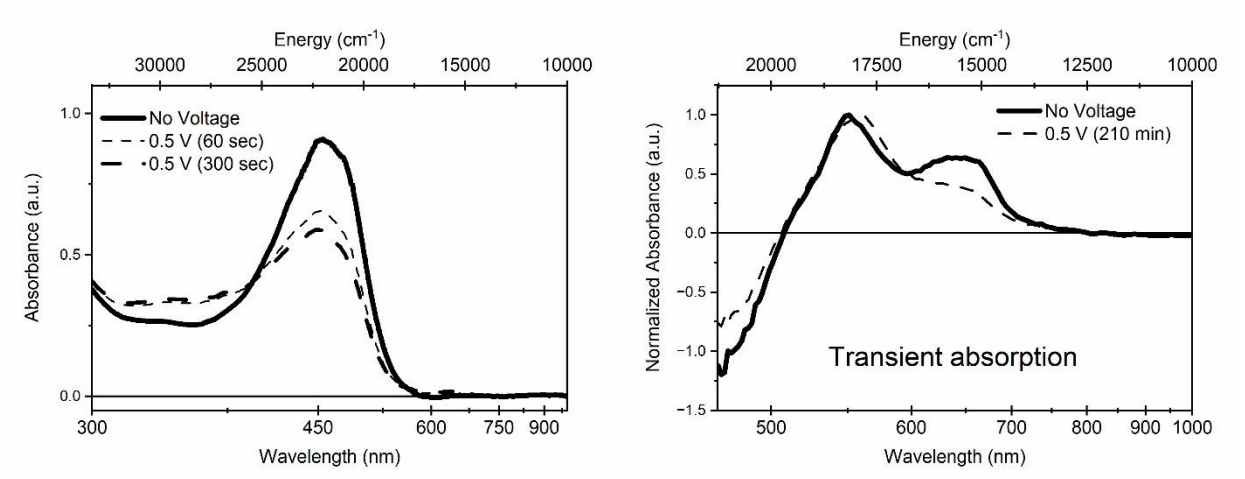
Changes of ICT State Dynamics of 8'-apo-β-Carotenal Induced by External Voltage in Electrochemical Cell

Gürkan Keşan[1], Emrah Özcan[1], Tomáš Polívka[1], Marcel Fuciman[1]

1 Faculty of Science, University of South Bohemia in České Budějovice, Branišovská 1760, 37005 České Budějovice, Czech Republic

The intramolecular charge-transfer (ICT) state is a typical excited state of carotenoids that contain carbonyl group in their conjugation. It becomes pronounced only in the polar environment and is strongly coupled to the S_1 state, forming a new electronic state, usually denoted S_1/ICT state. If present, it leads to decrease of the S_1 lifetime compared to nonpolar environments. For example, the S_1 state of carotenoid 8'-apo-β-carotenal has a lifetime of 25 ps in non-polar solvent *n*-hexane, but is reduced to 8 ps in polar solvents such as methanol or acetonitrile. However, the influence of applied voltage on ICT has not yet been investigated. Therefore, this work examines the influence of applied external voltage on the excited-state properties of 8'-apo-β-carotenal in acetonitrile by steady-state and ultrafast timeresolved absorption spectroscopy. The steady-state measurements showed that although the magnitude of the S_0-S_2 absorption bands decreased with applied voltage, their spectral positions and shape remain nearly the same. Comparison of pump-probe time-resolved measurements shows that the magnitude of the ICT band decreases during the experiment under applied voltage condition. The decrease of ICT band is also accompanied with a prolongation of the S_1/ICT state lifetime from 8 ps to 13 ps. Furthermore, turning off the applied voltage resulted in returning to no-voltage data within about 30 min. We have obtained satisfactory results to demonstrate that it is possible to tune the ICT state properties of carotenoids by applying an external voltage.



A highly photostable and versatile two-photon fluorescent probe for the detection of intracellular nitric oxide concentrations in macrophages and endothelial cells

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2 School of Chemistry, University of East Anglia, Norwich Research Park, Norwich, UK.

3 Departamento de Química Inorgánica y Orgánica, Universitat Jaume I, Castellón, Spain.

NO regulates physiological processes when present at low concentrations; however, at high levels, it is related to pathologies including cancer [1,2]. NO can be monitored using fluorescent probes, however, most of these are excited using ultraviolet or visible light, which results in poor penetration and high photodamage to cells. Alternatively, near-infrared light provides high photostability and tissue penetration, low level of autofluorescence and minimal photodamage upon long-term irradiation [3]. Although NO probes have been reported, the development of novel tools for the detection and quantification of NO is required for a better understanding of its role in biological processes.

The aim of this work is to develop a NIR excitable molecular probe for the intracellular detection of NO via a photoinduced electron transfer (PET) mechanism. The probe showed good sensitivity (LOD = 77.8 nM) and selectivity towards NO. Additionally, the fluorescence intensity of the probe was stable in a range of pHs from 4 to 9; and the detection of NO in acidic environments was successfully evidenced. The NO probe was able to detect NO in a variety of macrophages including RAW264.7 cells, by means of confocal microscopy (**Figure 1**) and multiphoton microscopy (NIR excitation) [4]. Following the great potential of the NO probe, a goldNP-based NO nanoprobe was developed by self-assembling a thiolated NO-sensitive ligand onto the surface of goldNPs showing excellent results in breast cancer and macrophages [5].

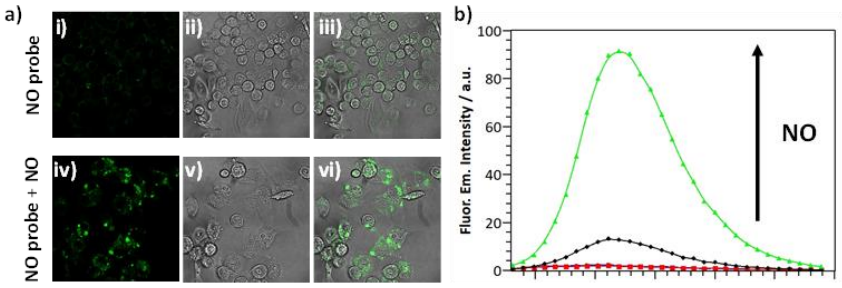

Figure 1. a) *Confocal microscopy images and* ***b)*** *intracellular fluorescence emission spectra of RAW264.7 cells treated with NO probe: i - iii) before (black) and iv - vi) after (green) stimulation of the cells to produce NO.*

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Plant circadian biology

Invited speakers:

IL39 David Somers (Columbus, USA)

The essential role of TOC1 phosphorylation in selective gene regulation

IL40 Chloe Zubieta (Grenoble, France)

It's just a phase: Structural characterization of LLPS and its role in temperature sensing in plants

IL41 Marian Nohales (Valencia, Spain)

Mechanistic insights into the circadian gating of the response to limiting light conditions in Arabidopsis

IL42 Colleen Doherty (Raleigh, USA)

Time and Stress: How Climate Change Impacts the Circadian Regulation of Stress Responses

The essential role of TOC1 phosphorylation in selective gene regulation

Jiapei Yan, David Somers [1]

1 Ohio State University, Columbus OH USA

Plant photoperiodic growth is coordinated by interactions between circadian clock and light signaling networks. How posttranslational modifications of clock proteins affect these interactions to mediate rhythmic growth and circadian period remains unclear. We have identified, and mutated, five phosphorylation sites in the Arabidopsis core clock protein TIMING OF CAB EXPRESSION 1 (TOC1) which eliminate detectable phosphorylation. The TOC1 phosphomutant (5X) fails to fully rescue the clock, growth, and flowering phenotypes of the *toc1* mutant. Further, the TOC1 phosphomutant shows advanced phase, a faster degradation rate, and poor binding at predawn hypocotyl growth-related genes (PHGs), leading to a net derepression of hypocotyl growth. NUCLEAR FACTOR Y subunits B and C (NFYB/C) stabilize TOC1 at target promoters, and this novel trimeric complex (NF-TOC1) acts as a transcriptional co-repressor with HDA15 to inhibit PIF-mediated hypocotyl elongation. (1)

In the context of circadian period, TOC1 phosphorylation is required for sustaining both circadian period and the robustness of rhythm. TOC1 binds to the promoters of two key clock genes, CCA1 and LHY, but only for *CCA1* binding is TOC1 phosphorylation required. Additionally, FAR-RED ELONGATED HYPOCOTYLS3 (FHY3), which normally activates CCA1 expression, interacts strongly with phosphorylated TOC1, which inhibits FHY3 action. In the absence of TOC1 phosphorylation, increased FHY3-mediated CCA1 activation results in shortened period and lower *CCA1* amplitude. Taken together, our results expand our understanding of post-translational regulation of both the photoperiodic control of hypocotyl growth and the circadian clock, illustrating how the regulation of growth and development occurs through selective interactions of specific co-factors with phosphorylated TOC1.

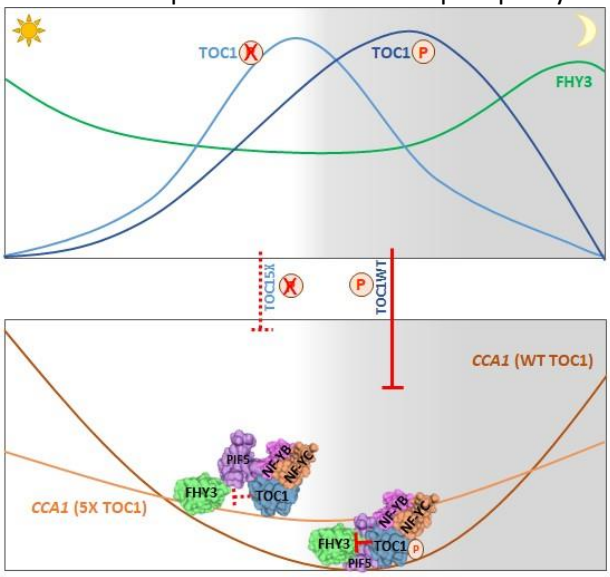

Figure. Proposed mechanism for the role of TOC1 phosphorylation in the control of *CCA1* oscillation. Early-phased 5X (unphosphorylated TOC1) increases earlier than wild-type TOC1 (TOC1 WT), but interacts poorly with FHY3, allowing *CCA1* transcription to increase. In WT, in contrast, lower FHY3 levels at night and strong inhibition of FHY3 activation of *CCA1* by phosphorylated TOC1 results in low CCA1 transcription, enhancing *CCA1* amplitude.

Reference

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It's just a phase: Structural characterization of LLPS and its role in temperature sensing in plants

Chloe Zubieta

Physiologie cellulaire et végétale, *Centre National de la Recherche Scientifique, Institut de Recherche Interdisciplinaire de Grenoble, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, Université Grenoble Alpes, France*

The increased average temperatures due to global warming have already altered plant phenology for both wild and domesticated species, presenting a critical challenge for food security in the coming decades. Plants are able to perceive even small changes in temperature and subsequently reprogram their growth and development for optimal reproduction and survival. However, the molecular mechanisms for this are still largely unknown.

We and collaborators have recently determined that EARLY FLOWERING 3 (ELF3), a core component of the circadian clock, acts as a direct thermosensor via liquid-liquid phase separation (LLPS). Using a combination of biophysical and structural techniques, we determined the dynamics and molecular mechanisms underlying the transition from the dilute to condensed phases in vitro. Based on these data, we explore how altering phase separation of the protein correlates with changes at the phenotypic level (hypocotyl length, plant morphology, flowering time) for Arabidopsis plants grown at different temperatures.

Mechanistic insights into the circadian gating of the response to limiting light conditions in Arabidopsis

Carlos Martínez-Vasallo[1], Benjamin Cole[2a], Jaime Pérez-Alemany[1], Javier Gallego-Bartolomé[1], Joanne Chory[3,4], Steve A. Kay[2] & Maria A. Nohales[1*]

[1] *Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas - Universidad Politécnica de Valencia, 46022 Valencia, Spain*

[2] *Keck School of Medicine, University of Southern California, Los Angeles, CA 90089, USA*

[3] *Plant Biology Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA.*

[4] *Howard Hughes Medical Institute, Salk Institute for Biological Studies, La Jolla, CA 92037, USA*

Present address: Department of Energy Joint Genome Institute, Berkeley, California, USA

Plants have a remarkable capacity to adapt to and cope with changes in environmental conditions. Because of the importance of light to their survival, plants have evolved sophisticated mechanisms to optimize responses to light. An outstanding adaptive response in terms of plant plasticity in dynamic light environments is the shade avoidance response which sun-loving plants deploy to escape canopy and grow towards the light. Under sunlight-night cycles, the plant's responsiveness to shade varies across the day, being maximal at dusk time. While a role for the circadian clock in this regulation has long been proposed, mechanistic understanding of how it is achieved is incomplete. Here, we provide mechanistic details directly linking a component of the circadian oscillator to the response to shade and show that it contributes to its temporalization by directly impinging on key transcriptional regulators of the light signaling pathway. In light of evolution and local adaptation, our findings give insights into a mechanism through which plants may have optimized resource allocation in fluctuating environments.

Work in the authors' laboratories has been funded by MCIN/AEI/10.13039/501100011033 (grants RYC2018-024108-I and PID2019-108577GA-I00 to J.G.-B. and PID2020-119491GA-I00 to M.A.N.), Generalitat Valenciana (grant CIDEGENT/2020/037 to M.A.N.), and the National Institute of General Medical Sciences of the National Institutes of Health (under award number R37 GM067837 to S.A.K.). J.C. is an investigator from the Howard Hughes Medical Institute. C.M.-V. is the recipient of a predoctoral fellowship from Generalitat Valenciana (CIACIF/2021/329). M.A.N. is a CIDEGENT Distinguished Researcher (CIDEGENT/2020/037).

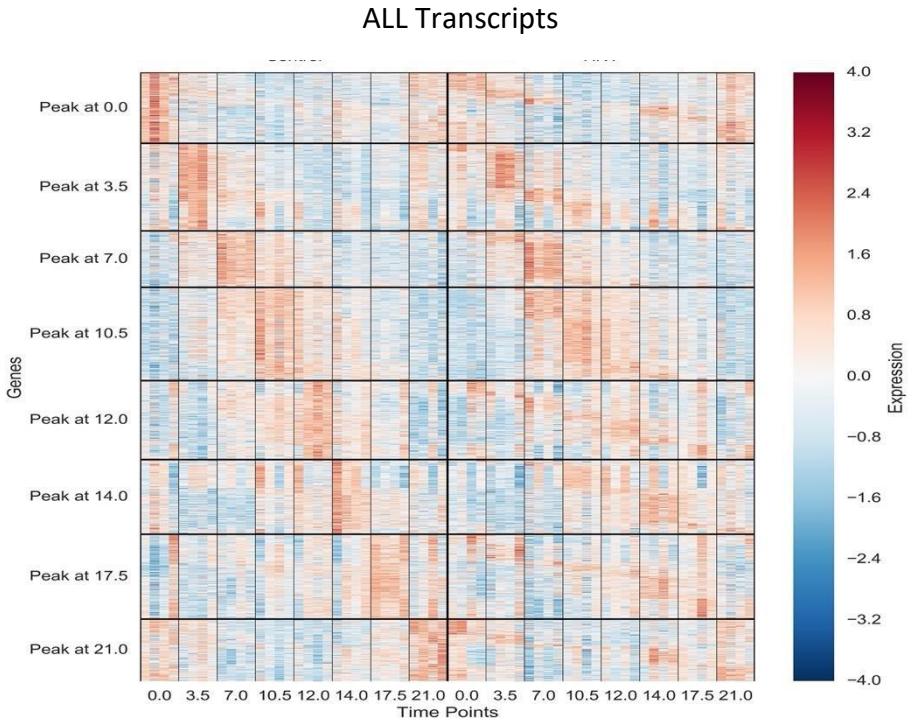
Time and Stress: How Climate Change Impacts the Circadian Regulation of Stress Responses

Kanjana Laosuntisuk[1] and Colleen J. Doherty[1]

1 Department of Molecular and Structural Biochemistry North Carolina State University

Endogenous circadian clocks coordinate the internal biochemical activities with recurring cycles in the external environment. The plant circadian clock is itself entrained by light and temperature cycles. This coordinated feedback provides a competitive advantage by providing a molecular mechanism to properly time daily and seasonal activities. As a result, many environmental responses are gated by the circadian clock, including biotic and abiotic stresses. We hypothesize that this allows the plant to conserve resources and reduce competitive cycles. However, one effect of climate change is a disruption of these daily and yearly rhythmic patterns, including earlier springs, off-season rain events, and changes in daily temperature cycles. The coordination of the circadian clock, established over evolution and breeding for optimal performance, is maladaptive to these new environments. Further complicating the effects of climate change, the entrainment cues provided by temperature cycles are disrupted by a more rapid rise in nighttime temperatures compared to daytime temperatures. This asymmetric nighttime warming already significantly impacts yield and grain quality in significant crops. We have examined the physiological, transcriptional, and metabolic responses to warmer nighttime temperatures. This work demonstrates that circadian disruption is a component of the adverse effects of warmer nighttime temperatures, identifies regulators and downstream targets that intersect between daily temperature cycles and the circadian clock, and explores the effects on biotic and abiotic stress responses.

Global gene expression coordination is disrupted by warmer nighttime temperatures (WNT)



all detectible transcripts from field-grown rice plants are organized row-wise by time of day of peak expression (red) in control plants. Columns are organized by the time of day the samples were collected. The coordinated expression observed in control plants is disrupted in plants grown under WNTs.

Desai, J. S., et al., (2021). Warm nights disrupt transcriptome rhythms in field-grown rice panicles. *Proceedings of the National Academy of Sciences*, *118*(25), e2025899118. https://doi.org/10.1073/pnas.2025899118

Flash presentations on plants and photosynthetic organisms

Flash presentations (see poster sessions for abstracts):

Jarne Berentsen: Optimizing Chloroplast Expansion Microscopy

Maria Agustina Dominguez Martin: Characterization of photosynthesis and photoprotection mechanisms in marine cyanobacteria

Paloma Lizondo Aranda: Nitrogen-Doped Indium Quantum Dots for plant-plant nutrient transfer

Luis Gustavo Teixeira Alves Duarte: De novo proteins as biogenic matrices for creating excitonically coupled dimers with charge-transfer character

Aneta Prochwicz: Characterisation of the impact of chloroplast avoidance on photosynthetic efficiency of plants in fluctuating light

Aleksandra Giza: Regulation of phototropin promoters from Arabidopsis thaliana

Pankaj Goyal: The phenomenon of light-responsive cellulose synthesis in bacteria

Yuna Mori: Development of chlorosome-type artificial photosynthetic antenna efficiently absorbing green light

Parallel symposia

Tuesday August 29

Afternoon

PDT and immunology

Invited speakers:

IL43 Lígia Catarina Gomes da Silva (Coimbra, Portugal)

Photodynamic therapy using LUZ51, a carboxamide halogenated bacteriochlorin, combines efficient cell killing with anti-tumor immunity

IL44 Gabriela Kramer-Marek (London, UK)

Photoimmunotherapy – the treatment to eradicate residual cancer cells and induce anti-tumour immune responses

IL45 Luis Arnaut (Coimbra, Portugal)

Photodynamic therapy and immune checkpoint blockers: How to work together

IL46 Pål Kristian Selbo (Oslo, Norway)

Photochemical internalization in combination with TLR3-based adjuvants improves vaccination effects

Ll47 Pål Johansen (Zurich, Switzerland)

Photochemical internalization of a tuberculosis vaccine for stimulation of T-cell responses: a mouse study

Oral communications:

OC62 Catarina Lobo: Targeting GD2 for Molecular Imaging and Photoimmunotherapy of Neuroblastoma

Flash presentations (see poster sessions for abstracts):

Nazareth Carigga Gutierrez: Increasing cancer permeability by photodynamic priming: From microenvironment to mechanotransduction signaling

Jimena Nicolás Morala: Rapamycin enhances Photodynamic therapy in cutaneous Squamous Cell Carcinoma

Patrycja Nowak-Sliwinska: Partial oxygen pressure prior and post AGuIXÂ® nanoparticles-based PDT in glioblastoma models

Photodynamic therapy using LUZ51, a carboxamide halogenated bacteriochlorin, combines efficient cell killing with anti-tumor immunity

Lígia C. Gomes-da-Silva[1], Mafalda Penetra[1], Bárbara Lima[1], Claire Donohoe[1], Luís G. Arnaut[1]

[1] *CQC – Coimbra Chemistry Center, University of Coimbra, Portugal*

Photodynamic therapy (PDT) is being used for the treatment of solid tumors, where the administration of a photosensitizing agent and light generate reactive oxygen species, which selectively damages the illuminated tissues. Most of the photosensitizers in clinical use have low absorption in the phototherapeutic window (650 to 850 nm) where tissues are more transparent to light. To overcome this limitation, we have recently developed a halogenated carboxamide bacteriochlorin named LUZ51 that exhibits a significant absorption peak at 739 nm, along with a high ROS quantum yield. Additionally, LUZ51 possesses a relatively small molecular weight and moderate lipophilicity (MM = 595.59 g/mol; log P_{ow} = 2.9) which was anticipated to enhance its interaction with the target cells[1]. In fact, *in vitro* studies demonstrated that LUZ51 is rapidly internalized by cancer cells, and upon photoactivation, it exhibits cancer cell-killing activity at concentrations within the nM range, even when short incubation periods were used.

Preclinical studies were conducted using two mouse models of cancer: Balb/c with subcutaneous CT26 tumors and Balb/c with orthotopic 4T1 breast cancer tumors. A protocol involving the intravenous administration of 0.2 mg/kg of LUZ51 followed by the delivery of 20 J/cm^2 induced strong anti-cancer effects, resulting in a cure rate of ~ 80% for Balb/c mice with CT26 tumors and ~ 40% for 4T1 tumors.

PDT is gaining increasing attention due to its immunomodulatory properties, which can effectively instruct the host immune system to recognize and effectively eliminate cancer cells[2]. Similar to other photosensitizers, LUZ51-based

PDT induced a strong edema, neutrophilia and increase in IL6 levels within the initial 24 h after PDT, indicating inflammation and activation of the innate immune system. Notable, 50% of CT26 tumor-bearing mice that were successfully cured with LUZ51-based PDT exhibited tumor rejection when rechallenged with live CT26 cells, suggesting the presence of adaptive immunity with immunological memory. This anti-tumor immune response is likely mediated by T cells, as immunocompromised mice (Balb/c nude), which lack both $CD8^+$ and $CD4^+$ T cells, failed to achieve complete tumor destruction upon PDT using LUZ51. Currently, the abscopal effects of PDT using LUZ51 are under investigation.

References

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Photoimmunotherapy – the treatment capable to eradicate residual cancer cells and induce antitumour immune responses.

Catarina Sousa Lobo[1], Chiara Da Pieve[1], Justyna Mączyńska[1], Florian Raes[1], Laura Privitera[2], John Anderson[2], Stefano Giuliani[3], Wojciech Kaspera[4], Gabriela Kramer-Marek[1]

[1] Division of Radiotherapy and Imaging, The Institute of Cancer Research, London, United Kingdom [2] Cancer Section, Developmental Biology and Cancer Programme, UCL Great Ormond Street Institute of Child Health, London, United Kingdom [3] Department of Specialist Neonatal and Paediatric Surgery, NHS Foundation Trust, Great Ormond Street Hospital for Children, London, United Kingdom [4] Department of Neurosurgery, Medical University of Silesia, Regional Hospital, Sosnowiec, Poland

Introduction: The dismal statistics of cancers like glioblastoma (GBM) and neuroblastoma (NB) result mostly from their distinctive tumour microenvironment (TME) and the incapacity to achieve a complete gross excision of the tumour mass during surgery. Therefore, in a clinical context a desirable outcome would be to selectively eradicate the residual or surgically inaccessible tumour cells and to restore the immunologically "cold" microenvironment of these tumours.
Near-infrared photoimmunotherapy (NIR-PIT) is a light-mediated targeted therapeutic approach that uses photosensitizers (e.g. IR700) conjugated to targeting vectors such as monoclonal antibodies (mAbs), antibody fragments or affibody molecules. If used intraoperatively, NIR-PIT could improve the extent of cytoreduction by providing an optimal delineation of resection margins. Furthermore, the photochemical processes triggered by the light-activated dye may lead to localised tumour cell-killing and activation of the tumour antigen-specific T cells locally in the TME.
Against this background, we postulated that PIT-targeting antigens like epidermal growth factor receptor (EGFR) for GBM and the ganglioside GD2 for NB, will not only help to define the tumour location and margins but additionally activate the host anti-tumour immune-response that could further enhance when combined with immune checkpoint inhibitors, reducing the GBM and NB recurrence.

Methods: The phthalocyanine dye IR700 was conjugated to $Z_{EGFR:03115}$ (EGFR-specific affibody molecule) and dinutuximab beta (anti-GD2 antibody). Cell viability, reactive oxygen species (ROS) production and major damage associated molecular patterns were studied in human and murine cell lines post-PIT using flow cytometry (FC), Western blot and ELISA. Xenograft and syngeneic murine tumour models (subcutaneous and orthotopic) were used to determine the therapeutic and tumour-specific immune response following PIT.

Results: We observed a significant decrease in cell viability in GBM cells expressing EGFR and NB cells with high GD2 levels in response to IR700-PIT. Generation of ROS post-PIT resulted in a translocation of calreticulin to the cell membrane and release of HMGB1, Hsp70 and ATP. PET, MRI and enhanced fluorescence signals confirmed the presence of well-defined tumour masses, and the ability of the conjugates to specifically target and visualise EGFR and GD2-positive tumours. Importantly, PIT led to a significant delay in subcutaneous GBM and NB tumour growth. Therapeutic efficacy of the affibody conjugate was observed in brain tumours as early as 24 h post-irradiation. In addition, tumour-infiltrating lymphocytes were elevated in both GBM and NB tumours post-PIT.

Conclusions: PIT is an attractive therapeutic strategy for tumours like GBM and NB. In both cases, following tumour resection in patients, PIT could i) contribute to the elimination of residual or surgically inaccessible cancer cells and ii) prompt the tumour-infiltrating cytotoxic T-cell immune response.

PDT and immune checkpoint blockers: How to work together

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Immunotherapies, and in particular immune-checkpoint blockade (ICB) therapy, have gained considerable clinical acceptance. It is estimated that nearly half of cancer patients in the United States are eligible for ICB therapies, but only 13% of them respond to such therapies [1]. This presents a challenge for the adoption of PDT in oncology, but it also offers an opportunity for PDT to enhance the response of patients to ICB therapies in view of the immune responses elicited by PDT [2]. This work reports the combination of redaporfin, which is a bacteriochlorin photosensitizer in clinical trials for head and neck cancer [3], with ICBs and discusses the requirements for sound combinations between PDT and ICBs.

PDT with redaporfin stimulates colon carcinoma (CT26), breast (4T1) and melanoma (B16F10) cells to display high levels of CD80 molecules on their surfaces. CD80 overexpression amplifies immunogenicity because it increases same cell (*cis*) CD80:PD-L1 interactions, which (i) disrupt binding of T-cells PD-1 inhibitory receptors with their ligands (PD-L1) in tumour cells, and (ii) inhibit CTLA-4 inhibitory receptors binding to CD80 in tumour cells. In some cancer cells, redaporfin-PDT also increases CTLA-4 and PD-L1 expressions and favourable combinations between PDT and immune-checkpoint blockers (ICB) depend on CD80/PD-L1 or CD80/CTLA-4 tumour overexpression ratios post-PDT. This was confirmed using CTLA4+PDT combinations to increase survival of mice bearing CT26 tumours, and to regress lung metastases observed with bioluminescence in mice with orthotopic 4T1 tumours [4].

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PCI combined with TLR3 adjuvants improve immune responses to peptide and protein-based vaccinations

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Synthetic peptides are promising candidates for therapeutic cancer vaccinations. However, a major problem in the development peptide-based cancer vaccines is insufficient priming of antigen-specific CD8+ cytotoxic T lymphocytes (CTL) due to low MHC class I presentation of antigen(s). To improve cross-presentation on MHC Class I, adjuvants are co-administered with the vaccine, and shown to enhance the immune response to vaccine antigens. In this work, we demonstrate that the endosomal escape technology photochemical internalisation
(PCI) strongly improve adjuvanted peptide- and protein-based vaccinations. PCI in combination with the toll-like receptor 3 (TLR3) agonist poly(I:C), resulted in a significant increase of antigen-specific CTLs. PCI + poly(I:C)-mediated vaccination with an E7-based synthetic long peptide, derived from the Human Papillomavirus 16 (HPV16), induced a robust activation of antigen-specific effector and memory CD8+ T cells and caused strong antitumor-responses in the murine HPV16 cancer model TC-1. PCI+poly(I:C) combination also enhanced CD4+ T-cell and IgG antibody responses against protein antigens (PPD, HBsAg and KLH). In conclusion, PCI combined with TLR3 adjuvants improve immune responses to peptide and protein-based vaccinations. Our PCI-based vaccine approach has a promising potential for the treatment of cancer and prevention of microbial infections.

Photochemical internalization of a tuberculosis vaccine for stimulation of T-cell responses: a mouse study

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Introduction: Vaccination against extracellular pathogens is efficient through production of pathogenspecific antibodies. However, infections caused by intracellular pathogens, such as Mycobacterium tuberculosis, are not easily treated by vaccination due to inaccessibility for extracellular antibodies and poor stimulation of cytotoxic T-cell responses. Photochemical internalization (PCI) is a method that can facilitate the cytosolic delivery of vaccine antigens to antigen-presenting cells (APCs), thereby stimulating MHC class I-restricted responses in CD8 T-cells. The aim of this study was to develop PCI as a method for delivery of M. bovis BCG for triggering of response of CD8 T cells with potential anti-tuberculosis effects. **Methods**: Mice received intradermal injections of live M. bovis BCG and photosensitizer TPCS2a. The photosensitizer was activated by administration of light 18 hours later. BCG-specific CD4 and CD8 T-cell responses were measured in blood, spleen, and lymph nodes by flow cytometry, ELISA, and ELISPOT.

Early inflammatory reactions were analysed in the skin by histology and immunohistochemistry. **Results**: PCI improved BCG-specific response of CD4 and CD8 T-cells, characterized by cellular proliferation and production of IFN-ɣ, TNF-α, IL-2, and IL-17. Light-activation of the photosensitizer was necessary for the improvement of the T-cell responses, and PCI improved antigen presentation in part by causing upregulation of MHC and costimulatory molecules on APCs. Histology of the skin showed local inflammation at the site of vaccination with strong involvement of neutrophils. **Conclusions**: These results demonstrate that PCI-based vaccination can be applied to live bacteria for targeted delivery of antigen to the cytosol of APCs. PCI enabled cross-presentation of the antigens for stimulation of antigenspecific CD8 T-cells, facilitating their proliferation and activation, improving the overall immunogenicity of M. bovis BCG. In the future, PCI may be important for the prevention of intracellular pathogens, and potentially also applied as immunotherapy of infectious diseases such as tuberculosis.

Targeting GD2 for Molecular Imaging and Photoimmunotherapy of Neuroblastoma

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Introduction: Neuroblastoma (NB) is the third most common paediatric solid cancer, which is associated with very poor survival. Many children are disabled by conventional high-dose therapies and eventually die from treatment-refractory metastatic disease. Although NB responds to radiotherapy, the widespread diseaseburden/marrow dissemination makes the disease detection challenging and the delivery of radiotherapy complex in the relapsed setting. Therefore, novel therapeutic strategies are urgently needed to improve the prognosis of NB patients. Immunotherapy targeting the ganglioside GD2, which is highly expressed on NB cells, is currently used in routine clinical practice. We postulated that anti-GD2 immuno-PET would enable real-time evaluation of GD2 expression for accurate patient selection to increase the efficacy of GD2-targeted therapies including near-infrared photoimmunotherapy (NIR-PIT) that is known to eradicate residual cancer cells and induce anti-tumour immunity.

Materials and Methods: Dinutiximab beta was radiolabelled with Zr-89 for PET studies (^{89}Zr-DFO-GD2) and conjugated to IR700 for NIR-PIT (GD2-IR700). The conjugates specificity of binding was assessed *in vitro* by protein binding-radioassay, flow cytometry (FC) and confocal microscopy on NB cells with different GD2 expression. The generation of reactive oxygen species (ROS) and phototoxicity were evaluated post-GD2-IR700 PIT. PET and biodistribution studies were performed up to 72 h post-injection of ^{89}Zr-DFO-GD2 in mice bearing human and murine NB tumours. Pharmacokinetics of GD2-IR700 and response to GD2-IR700-PIT were studied in NB mice models 24 h post-injection. Tumour samples were collected post-NIR-PIT for immunohistochemistry and FC analysis.

Results: *In vitro* binding studies confirmed specific and GD2-dependent targeting of ^{89}Zr-DFO-GD2. In NB mouse models, the radioconjugate successfully distinguished between tumours with different GD2 expression levels. In response to GD2-IR700 PIT, effective ROS generation led to a significant decrease in cell viability *in vitro* (Fig 1). *In vivo* PIT-treated tumours showed extensive oedema already a few hours after the treatment. Ki-67, CD31, H&E staining of tumour sections showed a reduced cell proliferation index, distinct differences in vessel density, extensive tumour necrosis and microhaemorrhage on the margins of treated tumours as early as 24 h post-PIT.

Conclusions: Further development of ^{89}Zr-DFO-GD2 and GD2-IR700 could lead to a non-invasive and novel image-guided therapeutic strategy targeting GD2 that could improve NB prognosis in the clinical setting.

Modulation of pigmentation and melanoma

Invited speakers:

IL48 Lionel Larue (Orsay, France)

Connecting Sex and Melanoma: CDH1 controls female tumor aggressiveness *(or The antagonistic role of β–catenin from melanoblast to melanoma)*

IL49 Markus Bohm (Münster, Germany)

Role of alpha-MSH-MC1R-mediated signalling in skin aging

IL50 Heather Etchevers (Marseille, France)

Non-inherited states preceding early-onset melanoma

IL51 Julien Ablain (Lyon, France)

Investigating the genetics of melanoma initiation and progression using zebrafish

Oral communications

OC63 Mouna Mhamdi-Ghodbani: Dermal stem cells as a model for the development of malignant melanoma

Connecting Sex and Melanoma: CDH1 controls female tumor aggressiveness (or The antagonistic role of β–catenin from melanoblast to melanoma)

Jeremy RAYMOND, Véronique DELMAS, Lionel LARUE

Institut Curie, Inserm U1021, CNRS UMR3347, 91405, Orsay, France

Cancer initiation and progression are sex, gender and age dependent processes. Melanoma, in particular, exhibits a higher incidence among women compared to men between puberty and menopause, making it the most gender-biased cancer during this phase. Our research highlights E-cadherin as a key factor in regulating tumor aggressiveness with respect to gender. By studying a mouse model of melanoma, we have successfully demonstrated that E-cadherin governs the formation of melanoma metastases in a sex-specific manner. Specifically, we have shown that E-cadherin inhibits the β-catenin/ERα/GRPR/YAP1 axis, which renders female melanoma cells responsive to estrogens and thereby more active in females. Activation of this axis promotes cell growth, clonogenicity, invasion, and resistance to anoikis, ultimately resulting in the formation of lung melanoma metastases in vivo. Interestingly, this axis has also been found to be active in other cancers, notably breast carcinoma. Our findings shed light on the role of E-cadherin in driving estrogen-dependent cancer and provide potential new therapeutic approaches for the treatment of female cancer patients."

Photopretection by the alpha-MSH-MC1R axis – is there a role in skin aging?

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Alpha-melanocyte-stimulating hormone (alpha-MSH)-mediated signalling via the melancortin-1 receptor (MC1R) is a key component of the cutaneous tanning response to ultraviolet (UVB) light. This physiologic response mediates photoprotection against UVBinduced genotoxic stress resulting in reduced apoptosis of epidermal cells by increased DNA repair. In individuals with the red hair pale skin phenotype loss of function (LOF) mutations of *MC1R* result in impaied alpha-MSH-MC1R signalling in melanocytes which explains their increased risk for melanoma. Interestingly, epidemiologic studies also suggest a link between alpha-MSH-MC1R signalling and dermal photoaging, the latter mainly mediated by UVA. To address the hypothesis as to whether alpha-MSH directly modulates UVA-mediated effects on senescence markers in dermal cells we used human adult dermal fibroblasts (aHDFs) expressing wild-type *MC1R* as confirmed by genotyping. Pretreatment (or cotreatment) of these cells with alpha-MSH (or related peptides such the superpotent NDP-alpha-MSH, the alpha-MSH-related tripeptide derivative KdPT or beta-endorphin) did not alter UVA1induced generation of intracellular reactive oxygen species. Alpha-MSH further had only minor effects on the time-dependent mRNA expression of p21, MMP1/3 and sirtuin 1 transcripts. Next, whole transcriptome analysis was performed on aHDFs carrying wild-type *MC1R* and LOF mutations of *MC1R*. Interestingly, even in absence of alpha-MSH marked differences were detected in the UVA-induced mRNA expression profile between cells expressing wildtype versus LOF *MC1R*, for example in genes of extracellular matrix structure and organization, intermediate filaments, polymeric cytoskeletal fibers, glycosamine binding or receptor regulator activity. Our findings provide a first base for further studies elucidating the role of MC1R in fibroblast biology and dermal photoaging.

Non-inherited states preceding early-onset melanoma

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Melanoma in childhood and adolescence lacks adequate preventive, diagnostic, and therapeutic strategies. A rare condition, its incidence is reported to be about 1.3-1.6 per million in children under 15 years of age and 15 per million in 15-19 y.o., with increasing incidence in adolescents by 4.1% annually since 1997. Pediatric melanoma is a distinct entity from melanoma in adulthood, which is clearly linked to ultraviolet radiation damage to the skin. Little is known about the observed progression from some benign melanocytic lesions to melanoma.

Large and giant congenital melanocytic nevi (GCMN) are also very rare, observed in an estimated 1 in 20-50,000 births. Unlike small CMN, they are heterogeneous, conspicuous skin tumors in terms of appearance, genetic background, and complications. As many as one of 20 children with a GCMN will develop cutaneous or extracutaneous melanoma before adulthood, with poor prognosis. This means that closer study of GCMN should provide clues not only into the early transformation events of melanoma in children, adolescents and young adults (CAYA), but also into the mechanisms of more common melanoma formation.

Like many other individually rare mosaic cutaneous disorders, young patients with GCMN have also unmet needs in terms of therapies, particularly concerning the syndromic forms that can have devastating effects in the central nervous system (kysts, tumors, epilepsies, hydrocephalus). I will present preliminary results implicating a specific cellular component of GCMN in vulnerability to tumor formation. This work, funded to date by patient advocacy groups, has been a model to organize a pan-European effort called MELCAYA, to boost research into these rare diseases and better understand the links between developmental biology, the effects of photon exposure and early steps of oncogenesis.

Reference

Etchevers HC, Dupin E, Le Douarin NM (2019) The diverse neural crest: from embryology to human pathology. Development 146(5):dev169821 doi: 10.1242/dev.169821.

Investigating the genetics of melanoma initiation and progression using zebrafish

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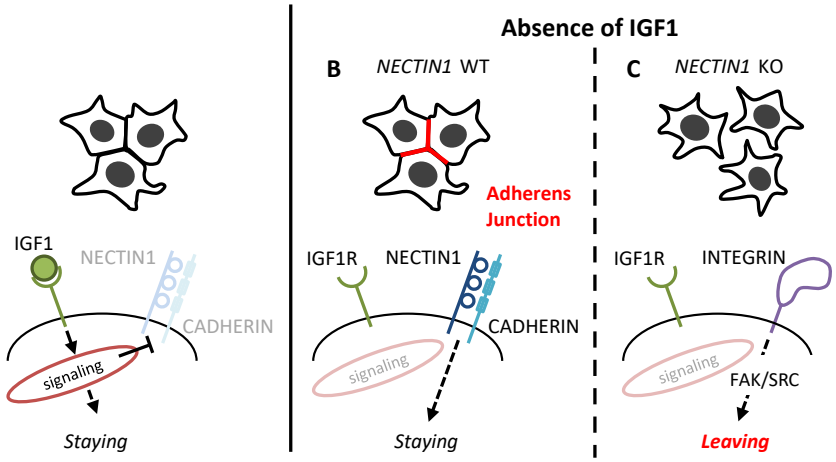
3 Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, USA.

Metastatic melanoma remains highly lethal despite the efficacy of targeted and immunotherapies. A better understanding of the mechanisms of melanoma initiation and progression is needed to develop new treatments. The analysis of cancer genomes offers a remarkable window on these mechanisms but the genetic complexity of melanoma has hindered the identification of new driver lesions. The zebrafish is uniquely positioned to unravel this complexity *in vivo*. We have developed genetic engineering tools to rapidly model human melanoma genotypes and study the function of candidate cancer genes in adult zebrafish. We thus identified *SPRED1* as a new tumor-suppressor, whose loss cooperates with KIT activation to accelerate melanoma initiation. In recent work, we found that the third most significant deletion in melanoma only contains the adherens junction gene *NECTIN1*, heterozygously deleted in 55% of cases. Staining of human melanomas revealed that NECTIN1 levels were lower in metastases than in primary tumors. To test NECTIN1 function *in vivo*, we generated *nectin1*-knockout melanomas in zebrafish. In a subcutaneous transplantation assay in transparent zebrafish where we can visualize the dissemination of pigmented cancer cells *in situ*, *nectin1*-knockout tumors spread 6-fold more than controls. Surprisingly, in human melanoma cell lines, *NECTIN1* inactivation by shRNA or CRISPR only increased cell migration and invasion upon depletion of IGF1. Mechanistically, IGF1 inhibition induced robust formation of adherens junctions between *NECTIN1*-wildtype melanoma cells, but not *NECTIN1*-knockout cells. Cell surface proteomics revealed that NECTIN1-deficient cells instead activated an integrin-dependent cell-matrix adhesion program resulting in FAK/SRC-mediated motility. Finally, in 20 human melanoma biopsies, adherens junctions were seen exclusively in areas of low IGF1, but not in NECTIN1-deficient tumors. Our study uncovers a mechanism by which the status of cell-cell contacts modulates the cellular response to local growth factor signaling, thus controlling the decision of cancer cells to stay or leave the tumor.



A Presence of IGF1Modelfor the role of NECTIN1 in melanoma. (**A**) In *NECTIN1* WT or KO the presence of IGF1, melanoma cells repress the formation of adherens junctions (AJs) and proliferate, independent of *NECTIN1* status. (**B**) In the absence of IGF1, *NECTIN1*-wildtype melanoma cells form robust AJs and rely on cell-cell adhesion to stay in the niche. (**C**) In the absence of IGF1, *NECTIN1*deficient melanoma cells –that are unable to establish AJs– switch to cell-matrix adhesion, and activate an integrin/FAK/SRC axis leading to their dissemination.

Reference

Ablain J, Al Mahi A, Rothschild H, Prasad M, Aires S, Yang S, Dokukin ME, Xu S, Dang M, Sokolov I, Lian CG, Zon LI. Loss of NECTIN1 triggers melanoma dissemination upon local IGF1 depletion. ***Nat Genet.*** 2022 Oct 13.

Dermal stem cells as a model for the development of malignant melanoma

Beate Volkmer 1, Christin Starzonek 1 , Mouna Mhamdi-Ghodbani 1 , Mohamed Said 2 , Rüdiger Greinert 1

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Introduction: Melanoma is the most aggressive and lethal form of skin cancer whose main etiological factor is exposure to ultraviolet (UV) radiation. The identity of the original target cell that acquires the requisite DNA lesions for transformation to melanoma still remains elusive. In human skin, neural crestderived precursors of melanocytes, the dermal stem cells (DSCs), are discussed to be at the origin of melanoma. DSCs are subjected to UV radiation, especially to UVA, which penetrates deep into the dermis, but also to UVB in the upper dermis layers. Therefore, DSCs are of great interest for the study of melanoma genesis. Although they are constantly exposed to solar UV radiation, it is still not investigated in detail how DSCs cope with UV-induced DNA damage.

Methods: Here, we report a comparative study of the DNA damage response in DSCs after UV exposure in comparison to fibroblasts, melanocytes and keratinocytes, all isolated from human foreskin. Cells were irradiated with different doses of UVA and UVB (using an irradiation device that mimics solar radiation) and DNA repair capacity, cell cycle alterations, as well as induction of apoptosis were measured. In addition, we investigated if DSC characteristics like the formation of spheres and the differentiation capability into melanocytes were affected by UV exposure.

Results: DSCs repaired DNA photolesions as efficient as the other skin cell types, with solely keratinocytes repairing significantly faster. Interestingly, DSCs showed neither great changes in cell cycle distribution nor induction of apoptosis following irradiation with both UVA and UVB. The other cell types displayed distinct alterations in terms of transient cell cycle arrests, especially after exposure to 900 J/m² UVB. Irradiation with 900 J/m² UVB also induced cell death in melanocytes and fibroblasts.

Following UV exposure, DSCs were still able to form characteristic spheres in suspension culture, however with a significantly lower amount of spheres when irradiated with UVB (300, 900, 3x300 J/m²). The size distribution of the formed spheres slightly shifted towards greater diameter. Differentiation of DSCs into melanocytes was not affected by single UV irradiation, as confirmed by expression analysis of melanocyte markers MITF, Tyrosinase, TRP1 and TRP2.

Conclusion: We demonstrated that the UV damage response of DSCs differed from other skin cells and that exposure to UV might also affect stem cell characteristics. Altogether, our results show that DSCs represent a novel and unique model to study the effects of UV radiation on the DNA damage response and differentiation ability of DSCs, connected with their potential relevance for the genesis of malignant melanoma.

Photosystem II and Oxygen Evolution

Invited speakers:

IL52 Anja Krieger-Liszkay (Paris-Saclay, France)

Plastid Terminal Oxidase (PTOX) protects photosystem I and not photosystem II against photoinhibition in Arabidopsis thaliana and Marchantia polymorpha

IL53 Roberta Croce (Amsterdam, The Netherlands)

Photoinhibition in PSII

IL54 Muhamed Amin (Groningen, The Netherlands)

Predicting the oxidation states of Mn ions in the oxygen-evolving complex of photosystem II using supervised and unsupervised machine learning

IL55 Noam Adir (Haifa, Israel)

Coupling isolated Photosystem II to Nano-photocatalysts for overall water splitting.

Plastid Terminal Oxidase (PTOX) protects photosystem I and not photosystem II against photoinhibition in *Arabidopsis thaliana* and *Marchantia polymorpha*

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The plastid terminal oxidase PTOX controls the oxidation level of the plastoquinone pool in the thylakoid membrane and acts as a safety valve upon abiotic stress, but detailed characterization of its role in protecting the photosynthetic apparatus in high light is limited. Here we used PTOX mutants in two model plants, the angiosperm *Arabidopsis thaliana* and the liverwort *Marchantia polymorpha*. *A. thaliana* possesses one PTOX isoform while *M. polymorpha* has two isoforms, one closely related to the isoform of plants and the second one closely related to the alga isoform (Messant et al., 2021). We created a uniformly green Arabidopsis PTOX mutant that expresses the bacterial carotenoid desaturase CRTI and a double mutant in Marchantia lacking the plant-type and the alga-type PTOX enzymes. In both species, lack of PTOX affected the redox state of the plastoquinone pool as shown by chlorophyll fluorescence. PTOX mutants showed less limitation of electron donation to photosystem I. Exposure of plants to high light intensity showed higher susceptibility of photosystem I to light-induced damage in the absence of PTOX while photosystem II was more stable demonstrating that PTOX plays both, a pro-oxidant and an anti-oxidant role *in vivo*. Our results shine new light on the function of PTOX in protection of photosystem I and II.

Reference:

Messant M, Shimakawa G, Perreau F, Miyake C, Krieger-Liszkay A (2021) Evolutive differentiation between alga- and plant-type plastid terminal oxidase: Study of plastid terminal oxidase PTOX isoforms in Marchantia polymorpha. Biochim Biophys Acta Bioenerg. 1862(1):148309

Photoinhibition in PSII

Roberta Croce [1]

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Predicting the oxidation states of Mn ions in the oxygenevolving complex of photosystem II using supervised and unsupervised machine learning

Muhamed Amin

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Serial Femtosecond Crystallography at the X-ray Free Electron Laser (XFEL) sources enabled the imaging of the catalytic intermediates of the oxygen evolution reaction of Photosystem II (PSII). However, due to the incoherent transition of the S-states, the resolved structures are a convolution from different catalytic states. Here, we train Decision Tree Classifier and K-means clustering models on Mn compounds obtained from the Cambridge Crystallographic Database to predict the S-state of the Xray, XFEL, and CryoEM structures by predicting the Mn's oxidation states in the oxygen-evolving complex. The model agrees mostly with the XFEL structures in the dark S_1 state. However, significant discrepancies are observed for the excited XFEL states (S_2, S_3, and S_0) and the dark states of the X-ray and CryoEM structures. Furthermore, there is a mismatch between the predicted S-states within the two monomers of the same dimer, mainly in the excited states. We validated our model against other metalloenzymes, the valence bond model and the Mn spin densities calculated using density functional theory for two of the mismatched predictions of PSII. The model suggests designing a more optimized sample delivery and illumiation systems are crucial to precisely resolve the geometry of the advanced S-states to overcome the noncoherent S-state transition. In addition, significant radiation damage is observed in X-ray and CryoEM structures, particularly at the dangler Mn center (Mn4). Our model represents a valuable tool for investigating the electronic structure of the catalytic metal cluster of PSII to understand the water splitting mechanism.

Coupling isolated Photosystem II to Nano-photocatalysts for overall water splitting.

Noam Adir

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Oxygenic photosynthesis is the most important solar-energy conversion (SEC) process in nature, providing the planet with its oxygen atmosphere and the biosphere with almost all of the carbon-based nutrients required for non-photosynthetic life. Photosynthesis has been studied extensively for the last century, leading to a deep understanding of the connection between the structures of the Photosynthetic complexes and their functionalities. The products of photosynthesis have been used as fuels for human society for thousands of years, however extensive combustion of these products has led to an environmental crisis. Non-combustion-based SEC by photosynthetic organisms and isolated systems is a potentially cleaner way to utilize the power of photosynthesis for this purpose. We have shown over the past few years the potential of using live photosynthetic systems for SEC[1]. Here, I will describe our success in functionally linking the water oxidation capabilities of isolated Photosystem II (PSII) to different materials, to perform SEC within different architectures of bio-photoelectrochemical cells (BPECs). In both cases there is the need to overcome the limited cross section for absorption of isolated PSII. In the first case we coupled thermophilic *T. elongatus* PSII to isolated phycobilisome complexes, leading to absorption and electron harvesting beyond the absorption of chlorophyll. Integration of the PBS–PSII super-complexes within an Os-complex-modified hydrogel on macro-porous indium tin oxide electrodes (MP-ITO) resulted in notably improved, incident photon-to-electron conversion efficiencies[2]. An alternative method is to couple PSII to gold-nanoparticles (AuNPs) to harvest electrical current[3]. Modifying these semiconductor AuNPs, with 2,6-Dichloro-1,4-Benzoquinone (DCBQ) efficiently and functionally enables very high currents to be obtained at wavelengths throughout the visible range. The AuNPs serve both as an antenna in the “green gap” as well as an improved conduit of the electrons to the BPEC.

References:

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2) Hartmann et al. J. Mat. Chem. A 2020

3) Shoyhet et al. J. Mat. Chem. A 2021

The impact on sunscreens on aquatic environments

Invited speakers:

Antony R. Young (London, UK) & Bernd Herzog (Grenzach-Wyhlen, Germany)

Introductory remarks

IL56 Sascha Pawlowski (Ludwigshafen am Rhein, Germany)

Environmental aspects of cosmetic UV filters used in sunscreens

IL57 Veronique Poulsen (Paris, France) and Antony R. Young (London, UK)
The impact of sunscreens on aquatic environments

Environmental aspects of cosmetic UV filters used in sunscreens

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[3] BASF Personal Care and Nutrition GmbH, Product Stewardship & EHS Data Management,

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Sunscreens and their UV filters within protect human skin from both skin damage and skin ageing. Therefore, sunscreens contribute to the EU skin cancer prevention program. UV filters are either organic or inorganic and adsorb and/or scatter the light in the UV A and/or UV B wavelength range. Due to various leisure activities such as swimming, snorkeling or diving, UV filters are likely to enter lakes, rivers and marine coastal areas. Due to their intrinsic physical-chemical substance properties (e.g., poor water solubility, high logarithmic octanol-water partition coefficient (log Pow)), UV filters may often be considered as harmful to the environment. In the EU, the consumer safety of UV filters is addressed via the Scientific Committee for Consumer Safety (SCCS), whereas the worker and environmental safety is addressed via EU REACH. Under REACH, hazardous UV filters require an environmental risk assessment (ERA) to demonstrate a safe use considering all life cycle stages. To cover the specific use in sunscreens, application-based ERA tools are developed through a joint collaboration between UV filter producers and cosmetic sunscreen formulators. Ongoing discussions related to the potential impact of UV filters on the global coral reef decline are jointly addressed through international working groups by developing standardized testing methods on corals. Additionally, the EcoSun Pass tool combines environmental and functional aspects of UV filters and therefore allows for the optimization of sunscreen formulations with respect to more ecofriendly products and thus also supports the EU Green Claims initiative.

The impact of sunscreens on aquatic environments

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Cosmetics and personal care products (CPCP) are most frequently used in the bathroom. After rinsing they flow down the drain where they mix with wastewaters. In industrialized countries they are directed to sewage treatment plants, but in many developing countries wastewaters may directly be discharged into rivers and the seashore. Among them, sunscreens may in addition be directly released by swimmers into lakes, rivers, and the ocean. UV filters, besides being part of sunscreens, are also present in a wide array of consumer and industrial products (such as paints and dyes), for the purposes of UV stabilization.

These products may in turn reach the waterways through urban and industrial wastewaters.

The report of UV filters presence in the environment, has led to a rapid increase in research on their potential environmental impact. An extensive review of Fate, Exposure, and Effects of Sunscreens in Aquatic Environments and Implications for Sunscreen Usage and Human Health has recently been carried out by the National Academy of Sciences at the request of the U.S. Environmental Protection Agency. It shows that there are gaps in the data set to be used in a robust risk assessment of sunscreens and their ingredients towards aquatic environments. Indeed, it is crucial to determine whether and under which conditions individual or mixtures of UV filters are a risk to organisms and ecosystems and where these conditions might occur.

To that end, their ecological risk assessment (ERA) proceeds by integrating information about exposures in the environment with information about adverse effects on aquatic species mostly observed under laboratory conditions. Many aquatic toxicity testing methodologies have been standardized (OECD, ISO, DIN, ASTM). Testing methods to assess the impacts of chemical compounds on corals have been developed by several stakeholders, but their standardization has yet to be undertaken. In parallel, the ICCS (International Collaboration on Cosmetics Safety) initiated a project for the development of exposure models to estimate concentrations of UV filters in marine and freshwater environments.

The purpose of this presentation will be to present the trends and advances in this challenging context.

Communications in photoprotection

Oral communications:

OC64 Jakob Heydenreich: Electronic UVR dosimeters used to measure time spent outdoors

OC65Beatriz Penin: Synthesis and photoprotection mechanism of novel sunscreens

OC66 Charareh Pourzand: The intracellular ‘labile ironome' as a predictor of the level of susceptibility of skin cells to sunlight: A powerful approach towards personalised sun protection

OC67 Diego Sampedro: Computational design and synthesis of new and efficient sunscreens

Flash presentations (see poster sessions for abstracts):

Batool Albadaineh: The dual protective role of trans-cinnamic acid as an antioxidant and iron chelator in UVAirradiated skin fibroblasts

Leonardo López: Design and evaluation of novel UV filters for photoprotection

Helena Polena: A randomized comparative study on melasma during summer with a visible light-protected tinted sunscreen versus a standard non-tinted sunscreen

Carolina Lorente: Antioxidant properties of vanillin during photosensitized oxidation of biomolecules

Electronic UVR dosimeters used to measure time spent outdoors

Jakob Heydenreich[1], Hans Christian Wulf [1]

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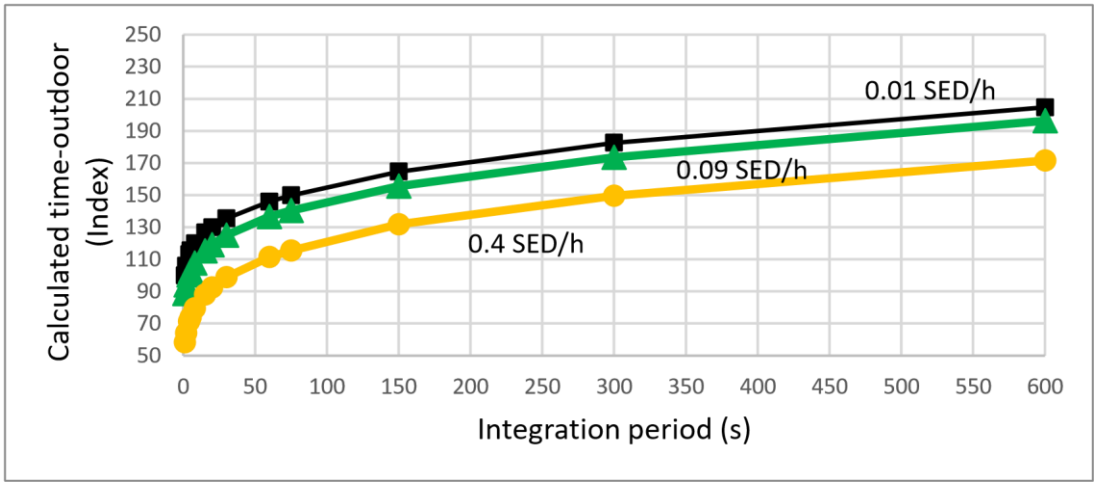
Personal ultraviolet exposure has for decades been measured with electronic dosimeters that at a given timepoint measures the solar ultraviolet radiation and saves the measurements with a timestamp. It can be used to establish relationships between behavior (hiking, working outdoor etc.) and sun exposure.

As there is no UV radiation (UVR) indoor one could assume that time with no UVR measurements equals being indoor. This can be used to calculate time spend outdoors and this method has been used in several papers. The method seems simple and straight forward but can cause significant errors.

In this study 8 people were wearing a personal electronic UVR dosimeter during a holiday with a total of 66 measurement days. Every second an UVR measurement was saved from 9 AM to 7 PM. The high 1second sample rate can be used to simulate lower sample rates by choosing a subset of measurements. Besides the sample rate, dosimeters can be programmed to save the average of the UVR measurements at different time intervals. These can also be simulated. The dosimeters sensitivity is defined as how low UVR levels they can measure, and different sensitivity levels can be simulated by setting recorded UVR measurements below the chosen level to zero. For example, can a dataset with a 1-second sample rate be used to simulate a 5 second sample rate by choosing every 5th measurement. If the measurements are saved every 10th minute, 120 measurements are summed (integrated) and measurements below the chosen sensitivity level is set to zero. Different dosimeter settings and properties can be simulated and thereby the effect on the calculated time-outdoors like shown on the figure below. Specific cases based on published dosimeter settings and the corresponding calculated time spend outdoors also are presented. One of the cases shows a difference of 86% using the exact same UVR exposure data.



The calculated time-outdoor is shown for different integration periods for all subjects (n=8) with an UVR measurement every second. An integration period is how often measurements are stored in the dosimeter's memory. Periods where the sum of all the measurements are zero are regarded as periods where the subject is indoor. The calculated time-outdoor is set to index 100 when the integration period is 1 second and the sensitivity is 0.01 SED/h (black curve). The green and yellow curve represent sensitivity 0.09 and 0.4 SED/h. It is seen that increasing the integration period (s) increases the calculated time spent outdoor. Lowering the dosimeters sensitivity decreases the calculated time spent outdoor.

Synthesis and photoprotection mechanism of novel sunscreens

B. Peñín[1], R. Losantos[1], L. López-Cóndor[1], D. Sampedro[1]

1 Department of Chemistry, Centro de Investigación en Síntesis Química (CISQ), Universidad de La Rioja, Madre de Dios, 53, 26006 Logroño, Spain.

Sunlight is essential for living organisms, although it also promotes photochemical reactions with harmful effects. In human beings, UV irradiation is the main responsible for these damaging effects causing permanent pigmentation and erythema which can evolve dangerously to cancer illness. Deleterious effects of sunlight may be minimized by the correct use of sunscreens. However, commercial sunscreens have also shown limitations and safety concerns.[1] Thus, the development of novel, efficient and safe photoprotection systems has become a crucial challenge for our society

In our research group, we have designed and synthesized a series of organic molecules, inspired by natural Mycosporine-like aminoacids (MAAs), which have excellent properties as sunscreens. These biomimetic molecules are based on an amino-cyclohexenimine core and exhibit high photostability, lack of fluorescence and strong UV absorption. Also, the synthetic organic protocol is readily accessible and scalable, making these structures ideal for real applications.[2]

Herein, we have synthesized a two different series of compounds which are classified based on the amine residues, which can be either an alkyl or an aryl group. Both families shared extremely good photochemical features such as high photostability and absorption but, according to our computational dynamic study of the photoprotection mechanism, we have demonstrated a two mechanism of operation: aryl-substituted compounds relax through a Z/E isomerization mechanism and alkyl-based compounds relax through a plane deformation of the ring core.[3]

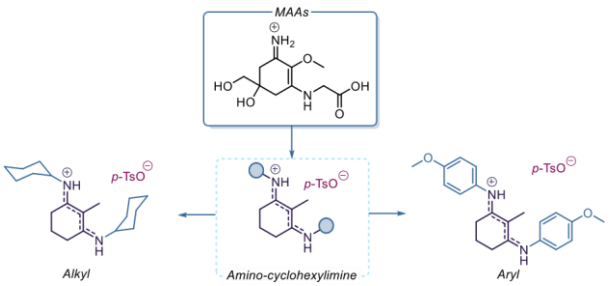


Figure 1. MAAs inspired derivatives.

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The intracellular 'labile ironome' as a predictor of the level of susceptibility of skin cells to sunlight: A powerful approach towards personalised sun protection

Charareh Pourzand[1,2], Haobo Ge[1], Batool Albadaineh[1], Agostino Cilibrizzi[2,3], Yongmin Ma[3], Robert Hider[3]

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2 Centre for Therapeutic Innovation, University of Bath, Bath, UK

3 Institute of Pharmaceutical Science, King's College London, London, UK

We have previously demonstrated that in skin cells, the level of cytosolic labile iron (LI) increases following UVA radiation, and this phenomenon plays a key role in exacerbating the oxidative damage in exposed cells leading to photoaging and photocarcinogenesis.[1] While originally LI was considered to be only cytosolic, there is now strong evidence that both mitochondria and lysosomes contain high levels of LI, making these organelles highly vulnerable to oxidative damage, notably following exposure of skin cells to oxidising UVA radiation that can be prevented by organelle-permeable/-targeted iron chelators pre-treatment.[2] However, to date the exact level of basal LI in skin cells' subcellular compartments have not been established. We have recently developed a series of highly specific fluorescent iron sensors based on hydroxypyridinones capable of sensitively evaluating the level of intracellular cytosolic, mitochondrial, and lysosomal LI in matched primary skin fibroblasts and keratinocytes obtained from the same individuals. These evaluations provided for the first time the intracellular 'labile ironome profile' of the skin cells, based on the highly specific sensors used and *ex situ* iron (II) and iron (III) calibration curves, as applicable. The results demonstrated that skin keratinocytes have a much lower level of intracellular LI than skin fibroblasts which is consistent with their higher resistance to iron-mediated oxidative photodamage exerted by UVA component of sunlight. In comparison skin fibroblasts from the same individuals exhibited two-threefold higher level of intracellular LI, with the major LI concentration accumulated in their mitochondrial compartments. The higher mitochondrial level of LI in skin fibroblasts correlated with their higher susceptibility to UVA-induced oxidative damage to mitochondria and necrotic cell death as evaluated by MTT and Annexin V-propidium iodide dual staining assays. The determination of the labile ironome of skin cells may be a powerful means of evaluating the extent of susceptibility of individuals' skin to photoaging and photodamage caused by sunlight.

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Computational design and synthesis of new and efficient sunscreens

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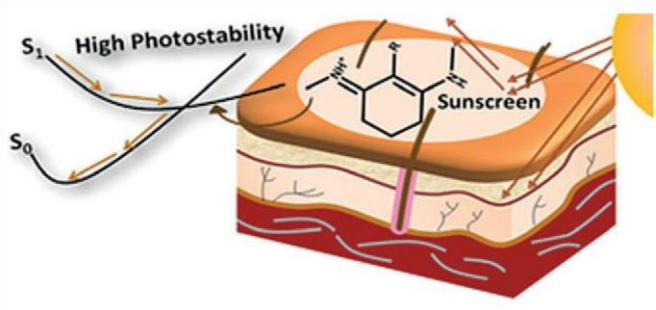
The incidence of skin cancer has been steadily increasing during the last years. This has been related to several reasons, being the increase in sunlight exposure time one of the most relevant. The use of sunscreens has proven of extreme importance in order to prevent the damaging effects of sunlight. There is currently a wide variety of commercial products available in the market, but in most cases the active ingredients that actually prevent the light absorption are only a few. Even more, some of them are suspected to have serious concerns about safety regarding both human body and the environment. On top of that, the commercial products are expected to be as environmentally friendly as possible, degrade after use and not bioaccumulate. This long list of requirements makes the preparation of new active ingredients for sunscreens a challenging task with profound implications in the field.

During the last years, we have explored by computational means a series of natural occurring compounds known to have excellent properties as sunscreens in biological environments. [1,2] Using the knowledge gathered by the computational study, we were able to design and prepare a series of compounds with very relevant properties. [3,4]

In this contribution, we will review our efforts in the computational design, preparation and use in real formulations of new compounds that could potentially act as active ingredients in sunscreens.



New sunscreens have been prepared after computational design and tuning.

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Parallel symposia

Wednesday August 30

Morning

Ultrafast studies to unravel the dynamics of photoactivated biological processes

Invited speakers:

IL58 Camila Bacellar (Villigen, Switzerland)

Ultrafast dynamics of biological systems at the Alvra endstation – SwissFEL

IL59 Nicoletta Liguori (Barcelona, Spain)

Realtime response of photoactive (macro)molecules to pH via a novel ultrafast spectroscopy tool

IL60 Sofia Kapetanaki (Pecs, Hungary)

Functional dynamics of a photoactivated adenylate cyclase revealed by ultrafast spectroscopy and time-resolved
X-ray methods

IL61 Miroslav Kloz (Prague, Czech Republic)

Structural changes of carotenoid echinenone in orange carotenoid protein (OCP) studied by femtosecond stimulated Raman spectroscopy

Oral communications:

OC68 Ines Camacho: Investigating a B12-Dependent, Photochemical Gene Switch by Ultrafast IR Spectroscopy

OC69 Pascale Changenet: Artefact-free balanced detection for measurement of subpicosecond circular dichroism in biomolecules

OC70 Agathe Espagne: Multi-scale transient absorption study of photohydration in the fluorescent protein Dreiklang and two point variants

OC71 Pavel Müller: Photoenzymatic decarboxylation of medium-chain fatty acids boosted by an unexpected auto-/co-catalytic effect of n-alkanes

OC72 Marten Vos: Ultrafast photoswitching in a flavo-enzyme involving a flavin-inhibitor charge transfer complex

OC73 Michelle Paulina Rademacher: Interactions between Angular Furocoumarins with DNA Studied by (TimeResolved) Spectroscopy

Ultrafast dynamics of biological systems at the Alvra endstation – SwissFEL

Camila Bacellar[1], Claudio Cirelli[1], Emma V. Beale[1], Florian Dworkowski[1], Philip J. M. Johnson[1]

1 SwissFEL, Paul Scherrer Institute, Villigen PSI, Switzerland

The advent of X-ray Free Electron Lasers (XFELs) has enabled the study of ultrafast phenomena in the X-ray range, due to XFFEL pulses' unique capabilities in terms of temporal and spatial resolution, as well as unparalleled peak brightness. Consequently, many established techniques have expanded into the time domain, while novel photon-intensive methods have been developed at XFELs. These advances have also made it possible to take full advantage of the element and site-specificity of X-ray spectroscopy techniques, such as X-ray Absorption (XAS) and X-ray emission (XES), as well as the spatial resolution from scattering and diffraction techniques, such as X-ray Solution Scattering (XSS) and Serial Femtosecond Crystallography (SFX), enabling novel insights into ultrafast processes occurring within complex biomolecular systems.

In this talk, I will introduce SwissFEL and the technical capabilities of the Alvra endstation and showcase scientific highlights from Serial Femtosecond Crystallography experiments, as well as present future perspectives in the field of ultrafast X-ray techniques applied to biological systems at Free Electron Lasers.

Realtime response of photoactive (macro)molecules to pH via a novel ultrafast spectroscopy tool

Nicoletta Liguori[1,2], Dominik Bäuerle[1], John T.M. Kennis[1]

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2 ICFO - The Institute of Photonics Sciences, Castelldefels, Barcelona, Spain

Changes of pH initiate a variety of molecular processes that are fundamental to life. It is known that, at the protein level, a sudden change of pH can drive conformational changes. In the case of proteins that bind chromophores, like photosynthetic light-harvesting complexes or microbial rhodopsins, pH-driven conformational changes then affect the energetic landscape of the pigments and, in turn, switch on and off the overall function of the complex[1-5].

However the generally (ultra)fast timescale (≤μs) associated to conformational changes is subresolution for the existing techniques, making it difficult to answer the question: how fast and via which intermediate steps do photoactive complexes respond to pH?

We will here present a novel ultrafast spectroscopy tool that finally allows to quantify: i) how fast conformational changes take place in photoactive materials following a sudden pH-jump and ii) how fast and in which terms the energetic landscape of these materials is affected when the environment changes.

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Functional dynamics of a photoactivated adenylate cyclase revealed by ultrafast spectroscopy and time-resolved X-ray methods

Jinnette Tolentino Collado[1], Zsuzsana Fekete[2], Gregory M. Greetham[3], James N. Iuliano[1], Sofia M. Kapetanaki[2], Mikhail Kozhaev[4], Matteo Levantino[4], András Lukács[2], Stephen R. Meech[5], Ildiko Pecsi[2], Katalin Pirisi[2], Kevin Pounot[4], Giorgio Schirò[6], Peter J. Tonge[1], Michael Towrie[3], Jovana Vitas[6], Martin Weik[6]

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2. *Department of Biophysics, Medical School, University of Pécs, 7624, Pécs, Hungary*
3. *Central Laser Facility, Harwell Science and Innovation Campus, Didcot, Oxfordshire, UK*
4. *European Synchrotron Radiation Facility (ESRF), Grenoble, France*
5. *School of Chemistry, University of East Anglia, Norwich, NR4 7TJ, UK*
6. *Institut de Biologie Structurale (IBS), Université Grenoble Alpes, CEA, CNRS, Grenoble, France*

Light activated proteins are at the heart of photobiology and optogenetics. OaPAC (photoactivated adenylate cyclase from *Oscillatoria acuminata*) is a promising candidate for optogenetic applications as the cAMP (cyclic adenosine monophosphate)-an important second messenger- levels produced in the adenylate cyclase (AC) domain of the enzyme can be modulated by blue-light. The hydrogen bonding network that surrounds the flavin in the BLUF (blue light using flavin) domain of the enzyme plays a critical role in sensing and communicating the changes in the electronic structure of the flavin to the protein matrix. Using pump-probe spectroscopy and time-resolved solution scattering in combination with site-directed mutagenesis to modulate the flavin environment, we have probed the protein dynamics of OaPAC and have mapped the photochemical mechanism and structural changes in the AC domain. Our work elucidates the direct connection between BLUF photoactivation and the structural and functional implications on the AC domain[1,2].

References

1. Jinnette Tolentino Collado, James N. Iuliano, Katalin Pirisi, Samruddhi Jewlikar, Katrin Adamczyk, Gregory M. Greetham, Michael Towrie, Jeremy R. H. Tame, Stephen R. Meech, Peter J. Tonge, and Andras Lukacs Unraveling the Photoactivation Mechanism of a Light-Activated Adenylyl Cyclase Using Ultrafast Spectroscopy Coupled with Unnatural Amino Acid Mutagenesis;

2. Sofia M. Kapetanaki, Jovana Vitas, Zsuzsanna Fekete, Ildiko Pecsi, Kevin Pounot, Matteo Levantino, Martin Weik, Andras Lukacs, Giorgio Schiro (in preparation).

Structural changes of carotenoid echinenone in orange carotenoid protein (OCP) studied by femtosecond stimulated Raman spectroscopy

M. Kloz [1], P. Čubáková [1] , T. Friedrich[2] , T.Polivka[3], E. Maksimov[4]

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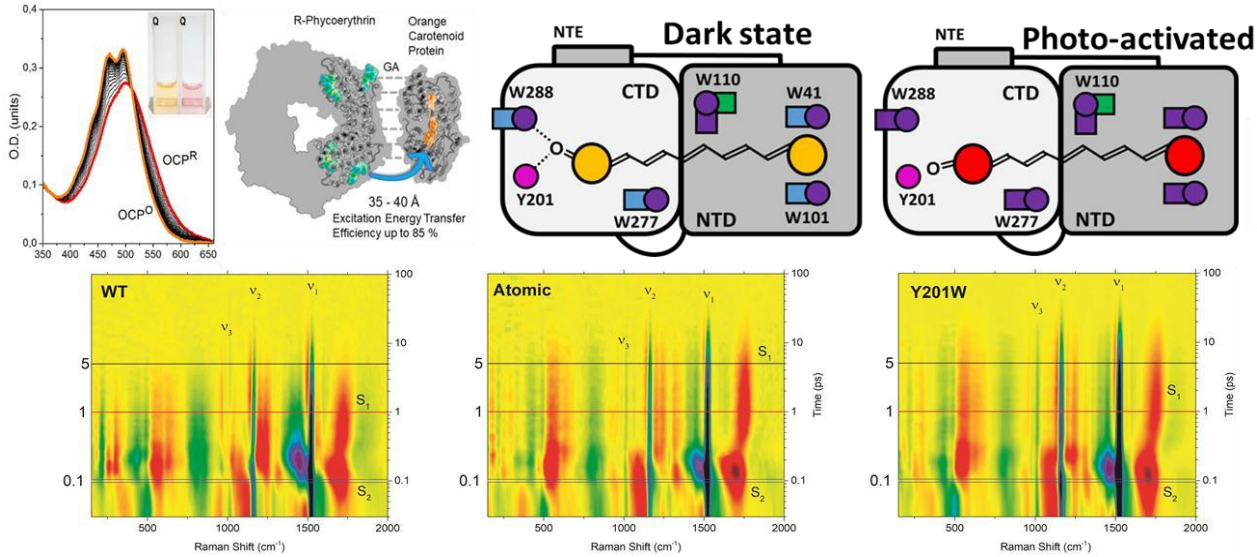
3 Institute of Physics, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

4 Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia

Orange carotenoid protein (OCP) [1] shown in the figure 1 is a perfect system to study changes in cofactor structure during photoswitching of proteins by Raman techniques. It hosts a single xanthophyll molecule and undergoes well-studied (but not yet fully understood) extremely low quantum yield photocycle that is associated with the loss of vibrational structure in the absorption spectra. Our experiments suggest that bond braking does not happen during the excited state.



The orange carotenoid protein consists of two subunits that get mutually loose after carotenoid excitation that switches it between the so-called "red" and "orange" states. In that form, it binds to other light-harvesting proteins while greatly increasing their non-radiative decay of excitons. Both the mechanism of OCP photoswitching and its subsequent role as a trigger of non-photochemical quenching is yet to be understood. We studied the wild type and two types of mutants (including utilization of non-canonical amino acids) to understand the role of hydrogen bond formation in the photoswitching mechanism by Stimulated Raman scattering. (figure is with courtesy of Eugen Maksimov)

Reference

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Investigating a B_{12}-Dependent, Photochemical Gene Switch by Ultrafast IR Spectroscopy

Ines Camacho[1], Emma Wall[2], Sam Hay[2], Neil Hunt[3], Alex Jones[1]

1. *Biometrology, Chemical and Biological Sciences Department, National Physical Laboratory, UK*
2. *Manchester Institute of Biotechnology and Department of Chemistry, The University of Manchester, UK*
3. *Department of Chemistry and York Biomedical Research, University of York, York, UK*

Although it has been known for decades that derivatives of vitamin B_{12} are sensitive to light,[1] it was only recently discovered that this photochemistry has a role in nature, with B_{12} the light-sensing chromophore in the bacterial transcriptional regulator protein, CarH.[2] In the dark, CarH binds to coenzyme B_{12} (5'deoxyadenolsylcobalamin, AdoCbl), which triggers tetramer formation and binding to DNA, thus blocking transcription (Fig. 1a). Photolysis of the upper axial ligand of AdoCbl (Fig. 1b) results in structural changes in the protein that lead to tetramer dissociation and DNA release.[3] Thus, light switches on the genes that control the biosynthesis of carotenoids in response to photooxidative stress.

The biochemistry and photochemistry of AdoCbl are strongly inclined towards radical mechanism. This appears to be very different in CarH, consistent with its role in protection against photooxidative stress. We have used UV-vis transient absorption[4] and time-resolved infrared experiments (unpublished) with femtosecond resolution to show how CarH tunes the photochemistry of AdoCbl, allowing it to function safely as a light-dependent transcriptional regulator. In these studies, we have identified charge transfer states that are stabilised by the protein and steer AdoCbl away from radical-based photochemistry. Further understanding of the link between specific protein-AdoCbl interactions and features of CarH's photochemistry will ultimately inform the rational design of optogenetic tools and light-responsive smart materials based on this system.

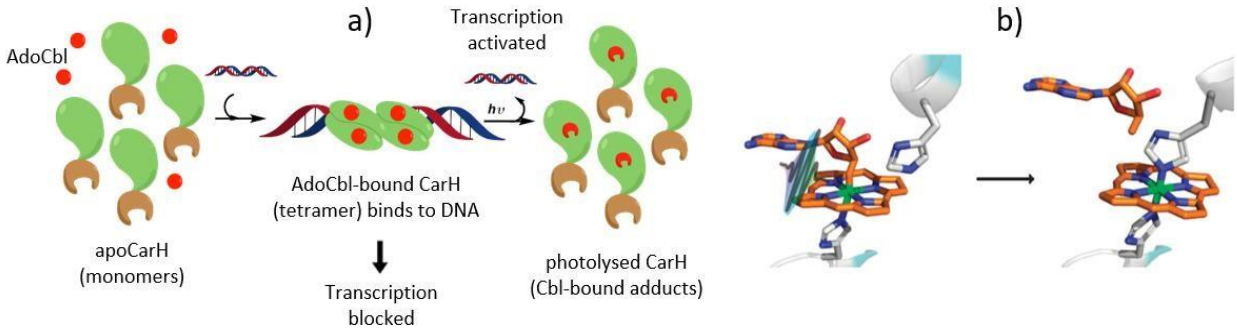

Fig. 1. a) *Protein-DNA complex assembly and disassembly;* ***(b)*** *AdoCbl photoconversion. Adapted from (1) & (4).*

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Artefact-free balanced detection for measurement of subpicosecond circular dichroism in biomolecules

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Circular dichroism (CD), which is the differential absorbance between left- and right-handed circularly polarized light, is a very popular technique for analyzing the secondary structure of biomolecules at equilibrium in solution. Combination of pump-probe techniques and CD spectroscopy provides a versatile tool to access the conformational and electronic structure changes of chiral molecules over a wide range of time scales. However, despite recent technological advances, time-resolved CD experiments at the femto-picosecond time scale are still challenging, due to their very weak signals prone to artifacts.[1] Taking advantage that the transmission of linearly polarized light by a chiral sample is elliptical, we recently implemented a dual-arm ellipsometry detection on a femtosecond pump-probe set-up, with the combination of a quarter-waveplate and a Wollaston prism (cf. figure). With this balanced detection geometry, the probe ellipticity can be directly accessed with a single laser shot allowing subpicosecond CD measurements with an accuracy of 30 □OD (1 mdeg) with very short acquisition times (*i.e.* a few min.).[2-3] In this presentation, I will illustrate the potential of this new detection and the strategy to eliminate polarization artefacts for the study of proteins and DNA.

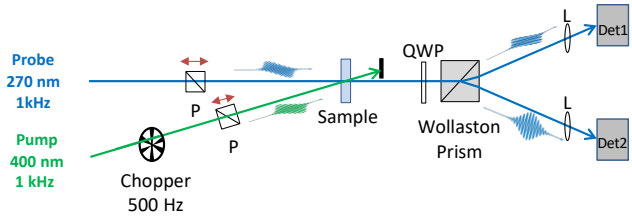

Figure: Sub-picosecond dual-arm ellipsometric TRCD detection. P: Glan polarizer. QWP: quarter-waveplate L: Lens. Det1and Det2: sample probe polarization detectors.

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Multi-scale transient absorption study of photohydration in the fluorescent protein Dreiklang and two point variants

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Dreiklang is a reversibly photoswitchable fluorescent protein used as a probe in advanced fluorescence imaging. It undergoes a unique photoswitching reaction based on the reversible addition of a water molecule to the chromophore, the mechanism of which is highly debated. In a first experimental study of Dreiklang by femtosecond transient absorption spectroscopy, we observed an ultrafast excited-state proton transfer (ESPT) which we assigned to the first photohydration step [1]. However, it was then proposed on the basis of quantum chemistry calculations that photohydration is initiated by an electron transfer from a tyrosine residue (Tyr203) to the chromophore [2,3]. Furthermore, beyond the primary step, the timescale and mechanism of formation of the final hydrated state remained to be established.

We will present a new study of the photohydration of Dreiklang by transient absorption spectroscopy in which 1) we covered for the first time all relevant timescales from 100 fs to seconds and 2) we compared the original Dreiklang protein with two point variants in which proton or electron transfer is blocked [4]. The picture that emerges from our work is that of a competition between photohydration and several non-productive reaction pathways (Fig. 1). We found that photohydration has a low quantum yield of 0.4%, that it proceeds via a short-lived charge-transfer (CT) excited state, as proposed by theoreticians, and that it is completed in 33 ns. Non-productive deactivation pathways comprise decay of the CT state by charge recombination, ESPT from the chromophore to a histidine residue (His145), or decay to the ground state via micro/millisecond lived intermediates.

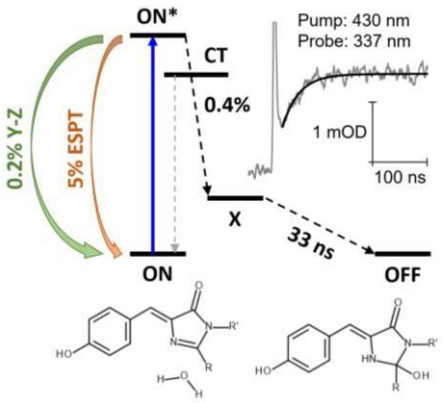

Figure 1. Proposed model for the light-induced dynamics of Dreiklang starting from its ON state, with chromophore structures in the ON and OFF states and experimental kinetics of formation of the hydrated OFF state.

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Photoenzymatic decarboxylation of medium-chain fatty acids boosted by an unexpected auto-/co-catalytic effect of *n*-alkanes

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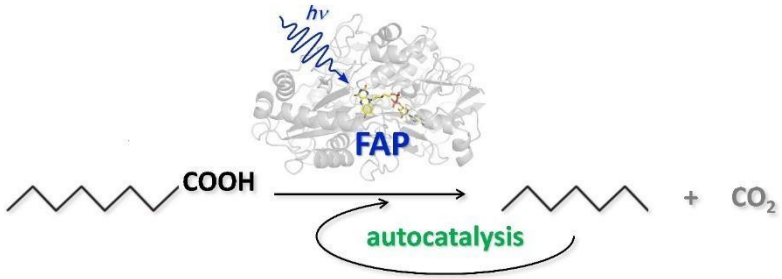
Ongoing climate change and geopolitical tensions are driving the search for renewable, carbon-neutral and local alternatives to fossil fuels. Photocatalytic conversion of fatty acids to hydrocarbons by Fatty Acid Photodecarboxylase (FAP) represents a promising route to green fuels. However, the alleged low activity of FAP on C2-C12 fatty acids seemed to preclude the use for synthesis of gasoline-range hydrocarbons. We reveal that *Chlorella variabilis* FAP (*Cv*FAP) *in vitro* can convert octanoic acid four times faster than hexadecanoic acid, its best substrate reported to date. We also show that *in vivo* this translates into a *Cv*FAP-based production rate over ten-fold higher for *n*-heptane than for *n*-pentadecane. Time-resolved spectroscopy and molecular modeling provide evidence that the high catalytic activity of FAP on octanoic acid is in part due to an autocatalytic effect of its *n*-heptane product. These results should guide future FAP improvement strategies and represent an important step towards a bio-based and light-driven production of gasoline-like hydrocarbons.



The product of octanoic acid decarboxylation, n-heptane, co-catalyzes the photodecarboxylation of further octanoic acid substrates by the FAP photoenzyme.

Reference: Samire P.P. *et al.* Autocatalytic Effect Boosts the Production of Medium-Chain Hydrocarbons by Fatty Acid Photodecarboxylase. ***Science Adv.*** **2023**, *9 (13)*, eadg3881.

Ultrafast photoswitching in a flavo-enzyme involving a flavin-inhibitor charge transfer complex

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Flavins are chromophores that display highly versatile redox properties. Some flavin binding proteins are functionally photoactive. Yet most display light-independent functions, although in these photophysical processes also occur. Such processes can have photoprotective functions, and may also be exploited for photocatalysis or photoswitching applications.[1] In this presentation we highlight the discovery of efficient and ultrafast photoswitching in a flavoenzyme-inhibitor complex,[2] and recent progress on this system. Monomeric sarcosine oxidase (MSOX) is a functionally nonphotocatalytic bacterial flavoprotein oxidase, which exhibits ultrafast photo-induced electron transfer in the semi-reduced form.[3] When complexed with the substrate-analogue inhibitor methylthioacetate (MTA) the oxidized FAD cofactor forms a charge-transfer (CT) complex strongly absorbing in the red spectral region, unlike noncomplexed FAD. Upon population of the photo-excited CT state, with near-unity quantum yield a state spectroscopically identical to the non-complexed enzyme is formed on the femtosecond timescale in a barrierless process. This implies that all CT interactions are vanished on this timescale. The initial CT complex is subsequently recovered in a thermally activated way on the nanosecond timescale. These are properties of a highly efficient red-absorbing photoswitch. Indeed, at cryogenic temperature the photochromic effect is quasi-permanent. The possible ultrafast structural changes associated with this unprecedented process are discussed, as well as recent in-depth characterizations of the process and possible extensions of this system for practical applications in the life sciences.

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Interactions between Angular Furocoumarins with DNA Studied by (Time-Resolved) Spectroscopy

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Linear furocoumarins, specifically psoralens, are eponymous for PUVA (psoralen + UV-A irradiation) therapy, which is widely used for the treatment of skin diseases such as psoriasis. Nowadays, angular furocoumarins (angelicins) are getting in the focus of biological studies [1], as they show two promising properties compared to psoralenes: After reversible intercalation between DNA bases, angelicins – contrary to psoralens, which only photo-react with thymine (T) bases – form photoadducts with T and cytosine (C) bases. Unlike psoralens, angelicins are postulated not to form cross-links within DNA after photoexcitation due to their angled structure. This ability to form only monofunctional adducts is associated with significantly reduced side effects of phototoxicity, which are disadvantageous in the PUVA therapy with psoralens [2]. Heretofore, angelicins have not been studied spectroscopically in detail.

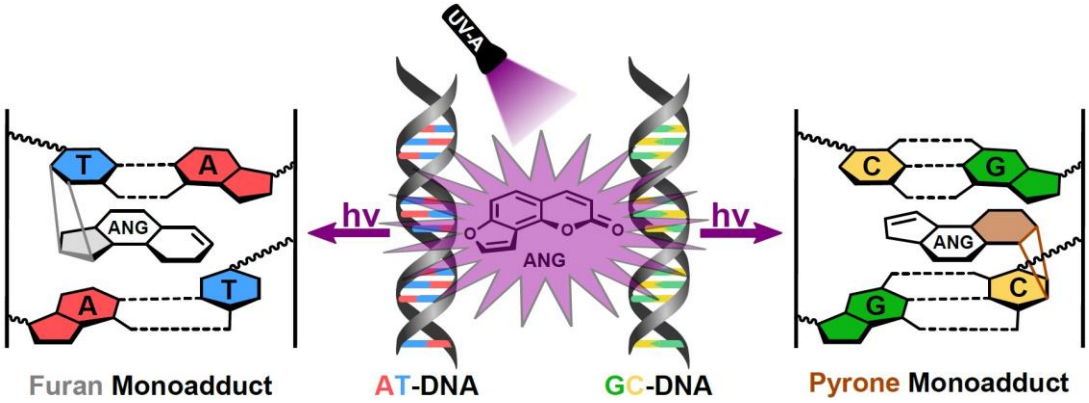

Figure 1: Interaction of angelicin (ANG) with DNA containing only adenine-thymine (AT) or guanine-cytosine (GC) bases. ANG intercalated in DNA forms UV-A light-induced furan monoadducts in AT-DNA. An analogous exposure experiment with GC-DNA yields pyrone monoadducts.

This ongoing study investigates the interaction of the parent compound angelicin (ANG, Figure 1, center) with DNA. Synthetic DNA containing only adenine-thymine bases (AT-DNA) or guanine-cytosine bases (GCDNA) is used. The selection of sequences is based on our pioneering work with psoralenes, in which timeresolved spectroscopy was used to observe photoinduced electron transfer (PET) in guanine-containing DNA [3]. Here, PET competes with the desired photoaddition [4,5]. Steady-state spectroscopy on ANG intercalated into AT- as well as GC-DNA confirms the postulated photoreactivity, with respect to T and C. Different double bonds of ANG are involved in the photoaddition with AT- (Figure 1, left) and GC-DNA (Figure 1, right). Preliminary time-resolved studies confirm the absence of a (fast) PET for intercalated ANG, which underscores the great potential of angelicins in phototherapeutic applications.

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Advanced technology for phototherapy

Invited speakers:

IL62 Ali Makky (Paris, France)

Phospholipid-porphyrin conjugates for the design of photoactivatable nanomaterials to combat bacterial infections

IL63 Gang Zheng (Toronto, Canada)

Photosensitizer nanoemulsions for anticancer and antimicrobial applications

IL64 Maria Rosa Antognazza (Milano, Italy)

Light-sensitive Nano and Microstructures based on Conjugated Polymers: Optical control of the Cell Fate

IL65 Giovanni Romano (Florence, Italy)

Emergent light source technology for antimicrobial phototherapy Oral communications:

OC74 Aixa Aguilera Garrido: Comparative study of indocyanine green and an indocyanine green dimer as photothermal agents

OC75 Ludovic Bretin: Testing ruthenium-based photoactivated chemotherapy on tumor-bearing mice models as a new treatment for uveal melanoma liver metastases

OC76 Huang Chiao Huang: A new photosensitizer nanoformulation for enhanced photodynamic therapy of brain cancer

OC77 Tassia Joi Martins: Supramolecular assemblies of fluorescent NO photoreleasers with ultrasmall cyclodextrin nanogels showing high photochemical performances

OC78 Sumiao Pang: Fluorescence-guided drug delivery and light dosimetry for PDT of ovarian cancer

OC79 Havva Funda Yagci Acar: Image-assisted Chemo/PTT combination therapy of Her2 (+) Breast Cancer utilizing Quantum dots: An in vitro and in vivo study

Phospholipid-porphyrin conjugates for the design of photoactivatable nanomaterials to combat bacterial infections

Louis-Gabriel Bronstein[1], Paul Cressey[1], Jana Alhoussein[1], Isokjon Pozilov[1], Abdelchakour Elkihel[1], Agota Toth[2], Florent Di Meo[2], Christophe Regeard[3] and Ali Makky[1]

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Phospholipid-porphyrin conjugates (PL-Por) are amphiphilic scaffolds that consist of porphyrin derivatives grafted to a lipid/phospholipid backbone [1-4]. Owing to their structural similarities with phospholipids, several PL-Por conjugates have shown to be able to self-assemble into liposome-like assemblies exhibiting unique photophysical properties compared to their monomeric counterparts. For these reasons PL-Por conjugates are considered nowadays as versatile building blocks to design supramolecular assemblies with multifunctional properties and thus their application in photodynamic therapy, photothermal therapy, photoacoustic imaging and photo-triggerable release properties [1, 4]. However, little is known about the impact of their structure on their 2D phase behavior at the air/water interface, their assembling properties, and their optical properties as well as on their photothermal and photodynamic activities.

In this work, we synthesized several PL-Por conjugates by changing either the photosensitizer (i.e. porphyrin derivatives or phthalocyanine) [5] or the lipid backbones. The conjugates self assembled into different supramolecular structures exhibiting tunable optical and photophysical properties. By combining a variety of experimental techniques with molecular dynamics simulations, we investigated the 2D phase behavior at the air/water interface, the thermodynamic, the optical properties and the structure of the PL-Por either self-assembled or when incorporated into lipid bilayer membranes [6]. Finally, the photothermal and photodynamic efficiencies of these assemblies were assessed on planktonic bacteria and their biofilms [7].

Our results demonstrated that whereas changing the porphyrin moiety controlled the packing of the monolayer and thus the formation of organized domains, the lipid backbone and/or the chain length dictated the structure of the formed domains. Finally, all of the conjugates were able to form supramolecular assemblies with bilayers structures and exhibit different photothermal and photodynamic activities against Gram + and Gram - bacterial planktonic cultures and their biofilms depending on their chemical structure.

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Photosensitizer nanoemulsions for anticancer and antimicrobial applications

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2 : Princess Margaret Cancer Centre

Novel nanoemulsions were created by the self-assembly of a photosensitizer salt shell encapsulating a food-grade oil core. This surfactant-free two-component nanoparticle system, named NewPS, has excellent colloidal stability, is amenable to different photosensitizer salt from porphyrin to indocyanine green (ICG), and is capable of co-loading with chemotherapeutics, antibiotics or CT contrast. When the NewPS consists of pyropheophorbide *a* mono-salt, it displayed a remarkable sub-nanomolar antimicrobial photodynamic therapy efficacy against planktonic bacteria and a 6-log reduction of bacterial burden in both biofilms and murine-infected wound model by improving photosensitizer delivery and homogeneous distribution in biofilm. When the NewPS consists of ICG, the complete conversion of ICG to dimeric ICG occurred on the nanoemulsion shell, followed by J-aggregation of ICG-dimer. This dimeric ICG-based NewPS is photobleaching-resistant, and it enables radiometric photoacoustic imaging to guide effective photothermal therapy. This flexible, yet simple nanoplatform can give rise to versatile theranostic applications for cancers and other diseases.

Light-sensitive Nano and Microstructures based on Conjugated Polymers: Optical control of the Cell Fate

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Use of light for selective and spatio-temporally resolved control of cell functions (photoceutics) is emerging as a valuable alternative to standard electrical and chemical methods. Here, we propose the use of smart materials, and in particular of organic semiconductors, as efficient and biocompatible optical transducers in the field of regenerative medicine.

Devices able to selectively and precisely modulate the fate of living cells, from adhesion to proliferation, from differentiation up to specific function, upon visible light will be presented. Examples of practical applications, recently reported by our group, include optical modulation of the activity of both excitable and non-excitable cells, control of essential cellular switches like transient receptor potential channels and other cationic channels, as well as effective modulation of intracellular calcium signaling for precise control of cell metabolic processes. We describe fabrication and optimization of micro- and nanostructured polymeric interfaces, in the form of beads and 3D scaffolds, with different cell models. As representative examples, we report on (i) functional interaction with intracellular proteins, leading to non-toxic modulation of the cell redox balance[1]; (ii) a novel strategy to gain optical control of Endothelial Progenitor Cell (EPC) fate and to optically induce angiogenesis in vitro[2]; (iii) optical modulation of mesenchymal stem cells and human-induced pluripotent stem cells physiological pathways[3]; (iv) effects of light-sensitive 3D scaffolds on neurogenesis[4]. Current knowledge about the photo-activated processes occurring at the conjugated polymer/living cell interface, obtained by complementing several physical/chemical/biological characterization techniques, is also critically discussed. The above mentioned study-cases represent, to the best of our knowledge, first reports on use of organic semiconductors for optical modulation of the cell fate, with disruptive perspectives in cell-based therapies. Future opportunities and perspective applications in regenerative medicine will be critically evaluated in the conclusions.

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Emergent light source technology for phototherapy

Giovanni Romano[1], Santi Nonell[2], Francesco Garzella[1], Giacomo Insero[1], Chiara Treghini[1], Gian Maria Rossolini[3], Lucia Pallecchi[4], Franco Fusi[1], Light4Lungs group[5], SAVES US group[6], EndoLight group[7]

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[3] *University of Florence, Department of Experimental and Clinical Medicine, Florence, Italy*

[4] *University of Siena, Department of Medical Biotechnologies, Siena, Italy*

[5] *S. Nonell, Institut Quimic de Sarria, Barcelona, Spain; G. Romano, Univ. of Florence, Italy; N. Henry, Sorbonne Université, Paris, France; M. Palomares, ICIQ, Tarragona, Spain; L. Menilli, Univ. of Padova, Italy; G. Taylor, Cardiff Scintigraphics Ltd, Cardiff, UK; A. Kadioglu, Univ. of Liverpool, UK; M. Manent, We-Do Projects, Barcelona, Spain.*

[6] *F. Rossi, CNR-IFAC, Sesto F.no (Florence), Italy; G. Toci, CNR-INO, Sesto F.no (Florence), Italy; F. Fusi, Univ. of Florence, Italy; P. Quaranta, Univ. of Pisa, Italy; S. Pollini, AOUC, Florence, Italy*

[7] *B. Orsini, Probiomedica srl, Florence, Italy; F. Rossi, CNR-IFAC, Florence, Italy; M. Seghi, P.C.E. Computer System srl, Pistoia, Italy; M. Biagini, University of Florence, Italy; A. Sechi, Valdisieve Hospital srl, S. Francesco (Florence), Italy*

In phototherapy, photons and photon delivery are fundamental ingredients to accomplish the therapeutic outcome. Therefore, innovation in phototherapy is also driven by innovation in light sources, which can reside in both technical advancements *per se* and in the way their emission is conveyed to the target [1].

Here, prototypes of new light sources are presented and discussed for their innovative content, together with preliminary data concerning their therapeutic efficacy. All the projects shown are focused on antimicrobial applications, the innovation being mainly in the source design to target difficult districts such as internal organs: (i) an ingestible and an endoscopic source for stomach infection control and eradication; (ii) a breathable light source in the form of a light-emitting aerosol to control lung infections [2]. In addition, a new UVC-based device is proposed for the suppression of viral and bacterial epidemic spread, in the form of a light barrier at □=222 nm combined with a vertical air flux to convey exhaled infected particles to the light source. This source is to be used in situations with constrained geometry (e.
g. public transportation, offices, waiting rooms etc.) in presence of humans, and is being tested with SARS-CoV-2 cultures and two bacterial strains of *Pseudomonas Aeruginosa* and *Staphylococcus Aureus.*

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Comparative study of indocyanine green and an indocyanine green dimer as photothermal agents

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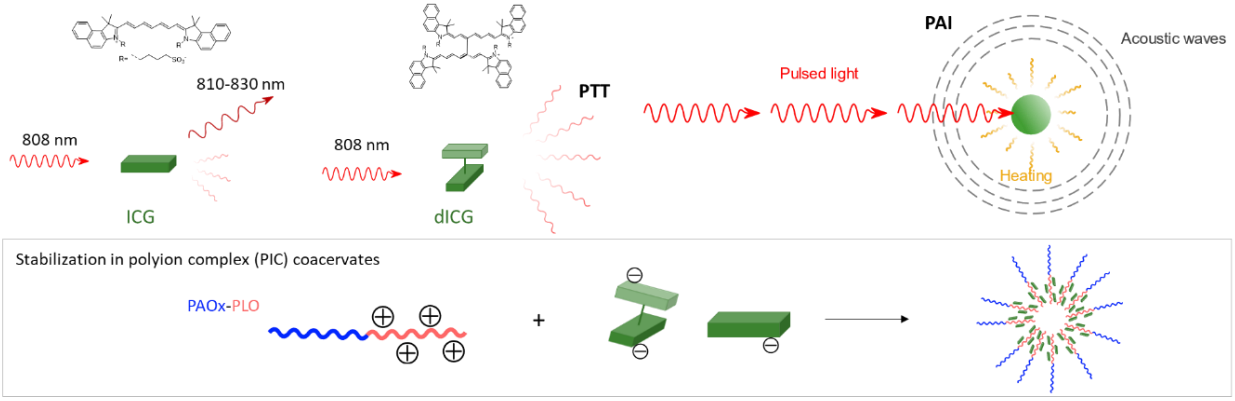
Indocyanine green (ICG) is a famous FDA-approved fluorescent marker used in medical diagnosis. ICG is characterized by an intense light absorption which fall within the in vivo 'transparent' window (700-900 nm), making it ideal for applications in photothermal therapy (PTT). The problem liked to ICG is its high photochemical and chemical instability and its very short effective half-live in circulation (120-240 s)[1,2] which sorely hamper its application in PTT. Within this work, we explored the potential of a covalent dimeric form of ICG (dICG) as PTT agent. dICG which could be efficiently prepared by light and oxygen-promoted ICG dimerization in water was studied in terms of optical and photothermal properties. dICG shows drastically reduced fluorescence properties compared to ICG, due to intramolecular resonance energy transfer. However, its photothermal conversion efficiency (PCE) is enhanced and its photobleaching propensity is reduced. The thermal expansion produced during PTT also generates photoacoustic waves which can be used for photoacoustic imaging (PAI), in a theranostic approach. Thus, the photoacoustic amplitude of ICG and dICG in solution was studied, reporting a better photoacoustic performance for dICG. *In vitro*, both molecules proved to be non-toxic in FaDu pharynx squamous cell carcinoma cells under dark conditions. Hence, our results reveal dICG as a promising PTT/PAI agent. However, as a small molecule, it biodistribution to tumour maybe be ineffective. Therefore, the nanoformulation of dICG was here investigated as an additional mean to improve dICG bioavailability. Both ICG and dICG molecules, were formulated in polyion complex (PIC) coacervates, using a double hydrophilic block copolymer built of a poly(2-alky)-oxazoline (PAOx) chain linked to a positively charged poly-Lornithine (PLO) segment. The electrostatic interaction between the PLO with the negatively charged ICG or dICG drove the formation of nanosized PICs. PICs were stable over time in water, PBS and in presence of increasing salt concentration, while maintaining the good performances in PTT.



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Testing ruthenium-based photoactivated chemotherapy on tumor-bearing mice models as a new treatment for uveal melanoma liver metastases

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Significance: Treatments approved for primary uveal melanoma (UM) are quite harmful to patients but very effective. However, 50% of primary tumors will metastasize mainly to the liver without efficient treatment yet, making this cancer quite deadly.[1] New clinical options are more than necessary. Photoactivated chemotherapy (PACT) is a new light-activated technology combining low systemic toxicity observed for photodynamic therapy, and the possibility of being activated in oxygen-poor tumors[2,3] while being useable for liver metastases through laparoscopy.[4,5]

Approach: The relevance of ruthenium-based PACT compounds for anticancer therapies in the context of UM liver metastases was investigated pre-clinically using subcutaneous and orthotopic mice models.

Results: Altogether our results show promising apoptotic photocytotoxicity and high antitumor efficacy, combined with rapid blood clearance and good biosafety *in vivo*.

Conclusion: These promising results *in vivo* highlight the potential interest of this new PACT technology for future clinical applications in UM liver metastases along with liver-related cancer in general.

Keywords: photoactivated chemotherapy, microtubule inhibitors, uveal melanoma liver metastases, tumor-bearing mice models, laparoscopic-assisted light irradiation

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A new photosensitizer nanoformulation for enhanced photodynamic therapy of glioblastoma

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Verteporfin is a promising photosensitizer that currently requires liposomal carriers for injection in glioblastoma patients. While liposome increases Verteporfin solubility, liposomes also significantly reduce the uptake and phototoxicity of Verteporfin in cancer cells. Here, we report a carrier-free, nanodrug of Verteporfin nanodrug (NanoVP) with a tunable size. The highly self-quenched NanoVP can be de-quenched in cancer cells for enhanced photochemical production of singlet oxygen. We also showed that NanoVP doubles photosensitizer uptake in glioblastoma cells compared to liposomal VP, enhancing tumor control and overall survival in mouse models. Lastly, we demonstrated NanoVP–PDT can safely open the blood-brain barrier, increasing drug accumulation in rat brains compared to using 5aminolevulinic acid. Overall, NanoVP is a new photosensitizer formulation and may facilitate treatment strategies for glioblastoma and diseases protected by the blood-brain barrier.

Supramolecular assemblies of fluorescent NO photoreleasers with ultrasmall cyclodextrin nanogels showing high photochemical performances

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Developing biocompatible NO photoreleasing nanoconstucts is of great interest in view of the large variety of biological role NO plays in many diseases, including cancer and infection, and the great advantage light offers in controlling NO release in space and time. In this contribution, we show that two recently developed hydrophobic NO photodonors (NOPD), **NBF-NO** and **RD-NO** (Figure 1),[1,2] can be effectively encapsulated in a recently developed ultrasmall nanogel (*ca* 10 nm in diameter) of □-cyclodextrins (□-CD) that is water soluble and shows superior inclusion affinity than native □-CD.[3] Both compounds preserve in the nanogel the nature of their photochemical properties individually and when co-entrapped, showing NO release regulated by blue and green light, associated to activatable green and persistent red fluorescence, respectively. The confinement in the nanogel leads to a significant increase of the NO photorelease efficiency of both compounds, probably due to the active role of the CD scaffold as reactant in the radical-mediated NO photorelease mechanism. Preliminary antibacterial tests ram- positive and -negative bacteria are also shown.

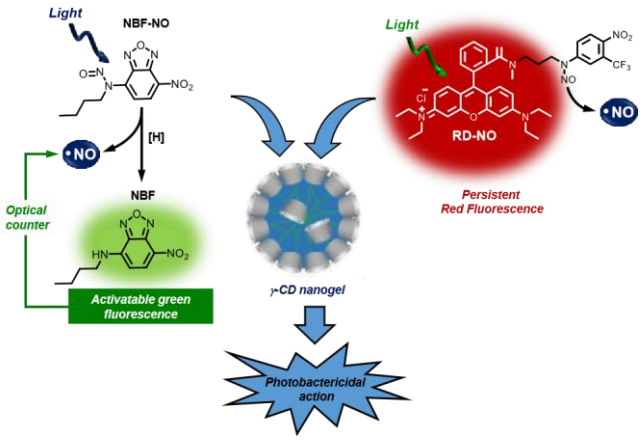

***Figure 1**. Molecular structure of **NBF-NO**, **RD-NO** and the γ-CD nanogel.*

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Fluorescence-guided drug delivery and light dosimetry for PDT of ovarian cancer

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Peritoneal micrometastases from ovarian cancer are challenging to detect and manage. To combat drug resistant micrometastases, new techniques that integrate targeted therapy, imaging, and treatment response monitoring are required. Over the years, considerable progress has been made in developing photodynamic therapy to treat peritoneal metastases. Still, it has yet to achieve complete responses or long-term tumor control. We have previously shown that combining fluorescence-guided intervention, targeted nanomedicine, and a cloud-connected medical laser platform (ML7710) improves the acute treatment response and reliability of photodynamic therapy for the management of micrometastases. This presentation will focus on a re-designed targeted, multi-agent nanoformulation for superior outcomes. The medical laser system has also been modified to simultaneously capture multispectral fluorescence emissions from the nanoconstruct compartments and perform fluorescence-guided PDT using a single optical fiber. We will also discuss the safety and animal survival results combining nanotechnology-assisted, fluorescence-guided PDT and standard of care chemotherapy in peritoneal metastases mouse models.

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Image-assisted Chemo/PTT combination therapy of Her2 (+) Breast Cancer utilizing Quantum dots: An *in vitro* and *in vivo* study

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Tumor-specific delivery of chemotherapeutic drugs via antibody-conjugated nanoparticles have been under heavy investigation to induce some locality to chemotherapy. Meanwhile, another highly local treatment method, photothermal therapy (PTT), may offer tremendous benefits when combined with chemotherapy. Integrating these two therapeutic approaches into one multifunctional nanoparticle is very attractive. Ag_2S QDs are ideal for such purposes with strong optical signal in the medical imaging window and strong light-to-heat conversion efficiency. In this talk, I will talk about such QDs delivering a Pt-drug and targeting HER2(+) SKBR3 cells in vitro and tumour xenografts in vivo, to induce PTT/chemotherapy combination for treating Her2(+) breast cancer. Selective accumulation of QDs in the cells and tumor was confirmed by fluorescence microscopy and IVIS imaging system. QD-based PTT was induced with an 808 nm diod-laser at doses that did not cause even redness on the skin of the nude-mice. The success of the combination therapy will be compared with PTT and chemotherapy alone.

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Ocular photochemistry: positive and negative effects

Invited speakers:

IL66 Joan Roberts (New York, USA)

Gene Replacement, Crispr, and Stem Cell Therapy for Retinal Damage

Il67 Malgorzata Rozanowska (Cardiff, UK)

Lutein and zeaxanthin - from retinal photoprotectants to photosensitizers

IL68 Tim Brown (Manchester, UK)

Defining healthy daily light exposure for humans and animals

IL69 Rigmor Baraas (Kongsberg, Norway)

The potential role of violet and blue light in eye growth.

IL70 Terje Christensen (Kongsberg, Norway)

Protection against occupational ocular hazards from optical radiation sources

Oral communications:

OC80 Ellen Bruzell: Eye protection against blue light photochemical hazard

OC81 Natalia Osik: Study of photoprotective biomolecules of the animal eye lenses

Gene Replacement, Crispr, and Stem Cell Therapy for Retinal Damage

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Loss of vision can be a result of genetic errors, age and/or environmental damage to the human retina. There are innovative technologies, recently developed: **Gene Replacement , Crispr, and Stem Cell Therapy** that can modify and possibly cure retinal damage.

Gene therapy is a technique where the genes are delivered through a vector virus administered by subretinal injection. This treatment has been used for improving retinal and visual function for treatment of patients with the *RPE65* -mediated inherited retinal disorders **autosomal recessive retinitis pigmentosa or Leber congenital amaurosis LCA.** type 2A. The treatment used recombinant adeno-associated virus serotype 2 (rAAV2) vector, altered to carry the human RPE65 gene (AAV2-hRPE65v2).

CRISPR i s a technology that precisely removes a mutant gene, while simultaneously adding the appropriate functional gene. There is a Crispr study in Phase I/II trials for improving vision loss in patients with LCA.type 10 (LCA10-IVS26). The mutation on the CEP290 gene that causes LCA.10 retinal degeneration is edited by subretinal injection of EDIT-101, a gene editing product.

Patient-derived **Stem cell replacement therapy is** currently in clinical trials for the treatment of Age Related Macular (AMD). RPE is a monolayer of cells located posterior to the photoreceptor layer and supports these rods and cones. Dry AMD at first damages RPE cells which progressively destroys the photorector cells resulting in the loss of vision. RPE replacement stem cells sheets are grown on biodegradable scaffold material. These stem cell patches are then surgically transplanted subretinally into an area alongside a border of geographic atrophy causing central vision loss. The Stem cell derived RPE can also be used as supportive cells to provide support for surviving rods and cones to slow down the progression of vision loss.

The retina of the human eye is uniquely suited for these therapies. It is accessible, self-contained (protected by the blood-ocular barrier) and is immune privileged. Progress or failure can be easily monitored with non-invasive imaging studies, including optical coherence tomography (OCT) and autofluorescence as well as standard ophthalmological examinations

Lutein and zeaxanthin – from retinal photoprotectants to photosensitizers

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Lutein and zeaxanthin are the only carotenoids of dietary origin which accumulate in the retina. They reach particularly high concentrations in the area responsible for acute vision where they can efficiently absorb incoming blue light, thereby protecting the outer segments (OS) of photoreceptive neurons and retinal pigment epithelium (RPE) from light-induced injury. Lutein and zeaxanthin are present in OS throughout the retina. Exposure of OS to the visible light leads to the release of all-trans-retinal (AtRal) from photoexcited visual pigments. AtRal is a potent photosensitizer, which upon photoexcitation in the presence of oxygen generates singlet oxygen, superoxide radical, and undergoes degradation into products, which are more (photo)cytotoxic than the parent compound. Carotenoids are well-known acceptors of energy from excited states of photosensitizers and singlet oxygen, and can also act as free radical scavengers. We have evaluated the bimolecular rate of scavenging by lutein and zeaxanthin of semi-oxidized AtRal and other retinoids present in OS and RPE - retinol and retinyl palmitate. Lutein and zeaxanthin effectively scavenge retinoid cation radicals with rates of $(0.7 - 1.1)\times10^{10}$ $M^{-1}s^{-1}$, and concomitantly form carotenoid cation radicals. OS are rich in polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), which are extremely susceptible to oxidation. Oxidized DHA (OxDHA) includes potent photosensitizers, which, upon photoexcitation with blue light, form a triplet state, singlet oxygen, and superoxide. We have shown that zeaxanthin is an efficient quencher of OxDHA triplet state but when included in liposomes containing OxDHA, it exacerbates phototoxicity to cultured RPE cells ARPE-19. Both lutein and zeaxanthin are susceptible to oxidation by light and/or iron ions. We have shown that oxidation of lutein leads to the formation of photosensitizers, which upon excitation with blue light generate a triplet state, singlet oxygen, and superoxide. We have measured the action spectrum of photooxidation of liposomes with degraded lutein and shown that the rates of oxygen consumption strongly increase with decreasing irradiation wavelength from 480 down to 320 nm. Exposure of ARPE-19 cells to blue light in the presence of oxidized lutein or zeaxanthin leads to cytotoxicity. This can explain why zeaxanthin on its own does not offer substantial protection from AtRal- or OxDHA-mediated phototoxicity to ARPE-19 cells but its protective effect strongly increases in the presence of vitamin E which prevents its oxidation.

Defining healthy daily light exposure for humans and animals

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Ocular light exposure has important and wide-ranging influences on human and animal health, through modulation of circadian rhythms, sleep, neuroendocrine and cognitive functions. The photoreceptive mechanisms underlying such actions are distinct from those supporting vision and hence established photometric measures do not adequately quantity the propensity of light to drive these 'non-visual' effects. Here I will discuss the development and validation of new approaches for quantifying light according to its non-visual effects, now codified as an SI-compliant international standard, and describe expert-consensus recommendations of healthy daily light exposure in humans based upon that approach. I will further discuss the development of a new wearable sensor to support large-scale tracking of real-world light exposure in relation to its non-visual effects. Finally I will cover ongoing efforts to extend these new measurement approaches established in humans to other mammals where the widespread use of (human) photometric measures neither appropriately captures visual nor nonvisual effects of ocular light exposure. In sum, these activities are intended to provide a framework to inform current lighting design and practice and to support future refinements in understanding the impacts of environmental lighting on human and animal health at both population and individual levels.

The potential role of violet and blue light in eye growth.

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The human eye is a complex organ. The eye of a newborn is about ¾ of its adult size and is typically hypermetropic (long sighted). Physiological eye growth and development continue postnatally until the end of adolescence whereby the refractive state reaches its optimum [1]. If physiological eye growth fails, the eye may grow too little and remain hypermetropic or grow too much and become myopic (near sighted). Neither is desirable, as either state requires prescription correction, and it can increase the risk of secondary eye disease later in life. In some parts of the world, myopia is considered an epidemic, whilst in other parts the prevalence of myopia appears to be stable. An identified difference is that where myopia prevalence is high, children are indoors during recess at school and spend little or no time outdoors in daylight, whilst the reverse is reported where myopia prevalence is low and stable [2]. These findings have been replicated in clinical trials; the introduction of outside time during recess to increase children's exposures to daylight appear to have successfully prevented myopia onset [3]. Daylight is broadband, containing all visible wavelengths, including violet and blue in varying relative amounts. Eye growth is regulated by retinal image processing and possibly circadian rhythmicity [4–7], affected by both the visual environment and genetics [2–6]. Thus, the intensity and spectral composition of daylight, combined with between-individual-differences in pigment (opsin) expression of cone photoreceptors and ipRGCs contribute to variable retinal processing and ocular circadian rhythmicity, respectively [4–7]. Physiological eye growth is desirable [1–4], and we will discuss the role of visible violet and blue light in this aspect.

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Protection against occupational ocular hazards from optical radiation sources

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The aim of using personal eye protection in environments where workers are exposed to optical radiation, is to avoid damage to different tissues in the eye. In most cases the protective equipment reduces the dose received by the eye to tolerable levels, i.e., below limit values. In European legislation (EU directive (2006/25/EC)) the limit values are those recommended by the International Commission on Non-ionizing Radiation Protection (1). However, ICNIRP guidelines can be overruled by national legislation.

Limit values are set to reduce or avoid a number of hazards, e.g. retinal thermal, retinal photochemical, thermal damage of the cornea, thermal damage of the iris, thermal damage of the crystalline lens and, for UV exposure, photokeratoconjunctivitis and cataract. For this purpose and for different wavelength regions, the limit values are expressed by several radiometric units as well as units calculated by weighting the spectral values of the radiometric units by various action spectra.

In this presentation we will give examples of standards for eye protection and analyse how standards for eye protection can contribute to compliance with the limit values.

The degree of eye protection for filters against exposure to UV in indoor workplaces as well as to solar radiation outdoors may depend on the luminous transmittance of the filters (2). Therefore, the time before the limit value is reached will vary depending on the choice of eyewear and the UVwavelength bands used in the indoor workplace or the time of day and latitude in outdoor workplaces. We will briefly present other standards than (2) for protection against optical radiation; assessment of doses and risks in different workplaces. A special case, protection against blue light hazard, will be discussed in greater detail in a separate presentation (3).

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Eye protection against blue light photochemical hazard

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The trend of wearing orange eyewear to filter out blue light may be caused by the awareness of how circadian rhythms affect sleep regulation. Currently, no internationally accepted limit values exist for the luminous exposure (lx˙s) or illuminance (lx) for which the eyes should be protected to avoid "circadian disruption". Thus, the optimal transmittance level of the non-visual eye protection filters will be unknown. Wearing orange eye protection filter glasses may increase the risk of accidents due to distortion of colour perception.

On the contrary, limit values exist for retinal photochemical damage, and a weighting function for blue light hazard is established (1,2). Dental personnel frequently use blue light emitting LED-based polymerization devices. Based on the blue light-weighted radiance and the use frequency, workday exposure to blue light can be exceeded (3), and we recommend using eyewear or shields to protect the eyes. However, transmittance values of eyewear intended for blue light exposure can vary by five orders of magnitude (4). Transmittance values can also vary for the same filter measured with different instruments. Thus, determining the optimal transmission for filters to be used when light curing dental materials as well as developing a reproducible method for transmittance measurement are warranted.

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Study of photoprotective biomolecules of the animal eye lenses

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Lens is a component of the eye system conducting and focusing light on the retina. It is permanently exposed to the UV light, which causes tissue damages through the ROS generation and the formation of highly reactive triplet species. Since the lens tissue mostly consists of enzymatically inert cells, lens protection relies on metabolites. For example, human lens is protected by UV filters (kynurenine and its derivatives) absorbing light in the UV-A region, ascorbate quenching triplet species, and glutathione scavenging photo induced radicals. However, the mechanisms of photoprotection in eye system of animals remain largely unsearched. Here we report the study of two photoprotective metabolites in the lenses of some birds and fishes revealed through quantitative metabolic profiling of animal eye lens.

We found ovothiol A (OSH), one of the strongest natural antioxidants, in fish lenses in surprisingly high concentrations. The study of OSH redox properties demonstrates the effectiveness of OSH-GSH couple with OSH neutralizing ROS and GSH maintaining ovothiol in reduced state. More importantly, OSH was found to quench the photoinduced triplet state of kynurenic acid with an almost diffusion-controlled rate constant. Thus, OSH is not only an excellent protector against oxidative stress, but it can provide a secondary photoprotection inhibiting the deleterious effect of solar UV irradiation on a lens tissue.

We also found for the first time that nicotinamide adenine dinucleotide reduced (NADH) is a molecular UV-filter of the bird eye lens. We discovered an unusually high NADH level in the lenses of some raptors and waterfowl. Photochemical measurements showed the reliability of NADH as a UV filter with strong absorption in the UV-A spectral region, high photostability, low yields of triplet state, fluorescence, and radicals under irradiation. NADH in the bird eye system protects the retina and the lens from photoinduced damages and improves the visual acuity by reducing chromatic aberrations.

Our findings, in addition to existing data, demonstrate the diversity of adaptations contribute to the eye protection in the animal kingdom. The investigation of animal tissues metabolome makes it possible to find such unique molecules as OSH, previously undiscovered in vertebrates, and to discover new facets of the biological functionality of well-known molecules like NADH.

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Advances on molecular mechanisms in natural and biomolecular biohybrids

Invited speakers:

IL71 Ardemis Boghossian (Lausanne, Switzerland)

Synthetic biology for enabling extracellular electron transfer in living microbes

IL72 Joanna Kargul (Warsaw, Poland)

Improving light harvesting and electron transfer pathways in biomolecular systems for solar conversion

IL73 Vincent Friebe (Munich, Germany)

In situ Time-Resolved Spectroelectrochemistry Reveals Limitations of Biohybrid Photoelectrode Performance **IL74 Nicoletta Liguori (Barcelona, Spain)**

The molecular origin of the OCP-dependent non-photochemical quenching mechanism in cyanobacteria

IL75 Margot Jacquet (Warsaw, Poland)

Improving biohybrid technologies using diazonium-based covalent molecular wiring strategy

Oral communications:

OC82 Sara Hernandez: Protein-promoted chromophore excited-state decay modulation

OC83 Dario Lacalamita: Anoxygenic Photosynthetic Bacteria-based Biocathodes for Green Hydrogen Production

OC84 Mary Wood: The role of eDNA in extracellular electron transport.

OC85 Maxime Kermarrec: Simulations of the Nucleosomal DNA : Mapping the Radical Cation Guanine

Synthetic biology for enabling extracellular electron transfer in living microbes

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Advancements in synthetic biology have pushed the frontier of materials science into the realm of engineering living materials. By integrating the tunability of nanomaterials with genetically modified living cells, biology can transcend traditional disciplinary boundaries to effectively address previously insurmountable challenges. This presentation highlights recent breakthroughs in the field of "living electronics," electronic devices that are based on living organisms that interface with nanoengineered scaffolds. In particular, this presentation focuses on bioengineering approaches for understanding and enabling biomolecular electron transfer mechanisms in light-harvesting microbes.

Building on recent discoveries in the extracellular electron transfer mechanisms of *S. oneidensis*, our research focuses on the heterologous expression of key cytochromes in a model facultative anaerobe (*E. coli*) and a photoautotroph (*Synechocystis sp.* PCC6803). We employ a combination of colorimetric and electrochemical techniques to characterize extracellular electron transfer in these engineered microbes. Interestingly, the bioengineered strains exhibit enhancements in both mediated and non-mediated extracellular electron transfer, with the extent varying with the specific combinations of expressed proteins. To optimize charge transfer in these cells, we have further developed novel approaches for interfacing the engineered microbes with modified electrodes and nanoparticles. Finally, we demonstrate the application of these modified *E. coli* and *Synechocystis* strains in living fuel cells and living photovoltaic systems for applications in wastewater treatment and light-harvesting energy, respectively.

Through a combination of synthetic biology and nanomaterials engineering, we have engineered microbes with a newfound ability to effectively transfer charge across their outer membranes. The research significantly broadens the range of exoelectrogenic microbes, unlocking new applications in microbial electronics across various fields such as biotechnology, environmental science, chemical synthesis, and even solar energy conversion.

Improving light harvesting and electron transfer pathways in biomolecular systems for solar conversion

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To efficiently capture the practically inexhaustible solar energy and convert it into green electricity and high energy density solar fuels provides an attractive 'green' alternative to the present-day fossil fuelbased energy systems, especially in the context of ever-growing global energy demand. This approach, dubbed 'artificial photosynthesis' carries great potential for the green energy transition from the centralised to decentralised systems for energy production.

In this lecture, I will show how the bottom-up rational design can yield the increased solar conversion efficiency and stability in biomolecular systems. The biophotocatalyst in these devices is the robust photosystem I (PSI) complex purified from extremophilic microalgae, then interfaced with various transparent electrode materials for production of green electricity and fuel. I will show that the performance of PSI-based devices can be greatly improved by tailoring the structure of the organic conductive interface to ensure the generation of unidirectional electron flow and minimisation of wasteful back reactions. Specifically, incorporating transitional metal redox centres together with plasmonic nanoparticles in the bio-organic interface significantly improves not only the light-harvesting functionality of the PSI photoenzyme but also increases its photostability and the overall photoconversion performance of the biomolecular devices. Such highly interdisciplinary and multifaceted rational design paves the way for generation of viable and sustainable technologies for solar energy conversion into fuel and other carbon-neutral chemicals.

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In situ Time-Resolved Spectroelectrochemistry Reveals Limitations of Biohybrid Photoelectrode Performance

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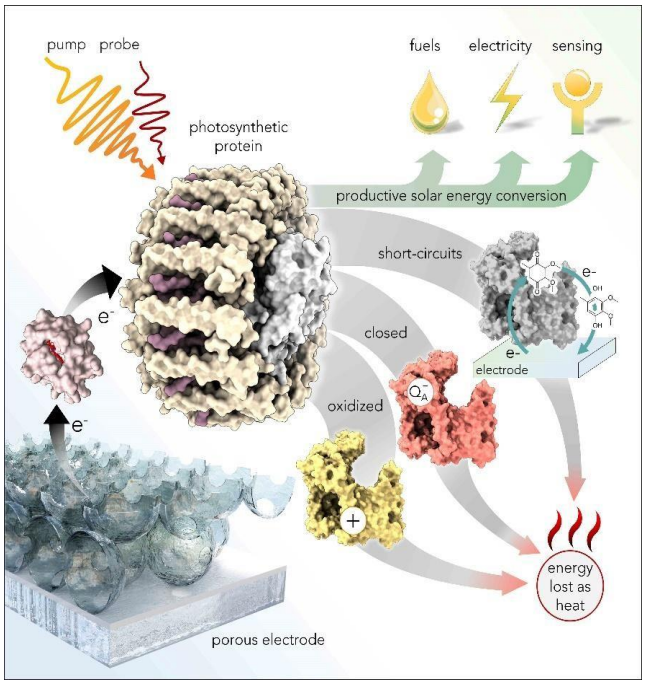
Abstract: Photosynthetic reaction centres catalyse the majority of solar energy conversion on Earth, and achieve this goal with a near-unity quantum efficiency. Capturing this high efficiency man-made electrodes is the goal of biohybrid technologies such as biophotovoltaics, biofuel cells, and biosensors. However, the removal of reaction centres from their natural cellular environment and their integration into an abiotic biohybrid architecture invariably introduces loss channels that compromise energy conversion efficiency. Here, we combined spectroscopy and analytical electrochemistry to identify electron transfer bottlenecks, back-reactions and short-circuits that affect the performance of a bacterial reaction centre-based biophotoelectrode. We determined that the system was over 90% efficient under low-Intensity light but dropped to ~11% efficiency under intense continuous illumination. Limitations and loss processes included bottlenecks in electron transfer that rendered 60% of reaction centres inactive, as well as a short-circuiting of 73% of the photochemical product from active reaction centres. These findings will help shape rational design strategies for improving the performance of biohybrid devices and extended to donor-acceptor type photocatalysts.



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Nawrocki, W. J.; Jones, M. R.; Frese, R. N.; Croce, R.; Friebe, V. M. In Situ Time-Resolved Spectroelectrochemistry Reveals Limitations of Biohybrid Photoelectrode Performance. *Joule* 2023, *7* (3), 529–544. https://doi.org/10.1016/j.joule.2023.02.015.

The molecular origin of the OCP-dependent non-photochemical quenching mechanism in cyanobacteria

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Cyanobacteria were the first microorganisms that released oxygen into the atmosphere billions of years ago. To do it safely under intense sunlight, they developed strategies that prevent photooxidation in the photosynthetic membrane. One of these strategies is accomplished by regulating the light-harvesting activity of their antenna complexes – the phycobilisomes – via the orange-carotenoid protein (OCP). This water-soluble protein encapsulates a ketocarotenoid and is photoactive. Under strong irradiance, OCP interacts with the phycobilisomes and triggers non-photochemical quenching (NPQ), a mechanism that safely dissipates overexcitation in the membrane. To date, the mechanism of action of OCP in triggering NPQ is unknown. We here applied ultrafast spectroscopy on the active domain of OCP bound to the phycobilisome core. Our results demonstrate that the binding to the phycobilisomes modifies the structure of the ketocarotenoid. We show that this molecular switch activates NPQ, by enabling energy transfer from the antenna pigments to the ketocarotenoid[1].

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Improving biohybrid technologies using diazonium-based covalent molecular wiring strategy

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The design of robust and cost-effective biohybrid materials requires rational chemical nanoengineering to afford a viable final device covering a wild range of applications including artificial photosynthesis, biophotovoltaic, and biosensing. A key factor for the optimal performance of biohybrid devices is to ensure efficient electronic communication between the biocatalysts and the electrode surface together with the appropriate orientation of the (photo)electroactive protein towards the electrode to achieve the highest possible charge transfer efficiency and minimize wasteful back reactions.

Here, we present the development of covalently linked metalorganic wires on two transparent and cheap electrode materials: fluorine-doped tin oxide (FTO) and FTO/single-layer graphene (FTO/SLG). The wires are terminated with nitrilotriacetic acid (NTA) metal complexes which serve as universal molecular anchors to immobilize in an oriented manner His_6-tagged proteins, such as biophotocatalysts and other types of redoxactive proteins of great interest. We show that the covalent functionalization of the two different electron-rich surfaces leads to the formation of the molecular wires that promote the *p*-doping resulting in a significantly enhanced unidirectional cathodic photocurrent up to 1 $\mu A \cdot cm^{-2}$. Density functional theory modeling reveals that the high photocurrent values are due to two distinct mechanisms of electron transfer originating from different orbitals/bands of the diazonium-derived wires depending on the nature of the chelating metal redox center (Co^{2+} or Ni^{2+}). We further employed these novel metalorganic interfaces for the construction of biophotovoltaic systems incorporating His_6-tagged cytochrome *c* (a natural electron relay) and photosystem I. We identified an additional doping effect in thus constructed metalorganic interfaces essential for achieving more effective vectorial electron transfer. The doped nanoarchitectures depict much-higher photocurrent outputs compared to the undoped analogs, and faster photo-induced electron transfer kinetics compared to those obtained for the systems in the presence of freely-diffusive redox mediators. These results open the way for the development of better-performing and sustainable biohybrid technologies by removing the critical bottleneck of using toxic and diffusive redox mediators.

Jacquet M.* *et al*., *Chem. Mater*., **2022**, 34, 3744-3758 ; Jacquet M.* *et al*., *in preparation*

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Protein-promoted chromophore excited-state decay modulation

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In natural photosynthesis, sunlight harvested energy transfers through a dynamic protein-chromophore network with a remarkable near-unity quantum efficiency. Local fluctuations in the protein structures and conformational changes are essential for nature's efficient light conversion, but their design principles remain to be elucidated.

In our lab, we develop simplified hybrid systems linking chromophores to engineered proteins. This strategy allows us to rationally modify the protein-chromophore interaction's nature and strength, evaluated as bio-exciton coupling. In particular, in this work, we link pyrene chromophore to modified alpha helix proteins by specific linkers. We study pyrene excited state dynamics using time-resolved spectroscopy and correlate excited decay kinetics with protein´s structural changes using NMR and molecular dynamics. In the protein, pyrene show multiple decay channels, which are not observed when pyrene is in solution (Figure 1). Interestingly, changes in the protein´s backbone induced by specific amino acids make pyrene excitation follow a preferential decay pathway. Our study aims to develop protein-based bio-hybrids which use controlled protein dynamics to improve light energy conversion in biomimetic systems.

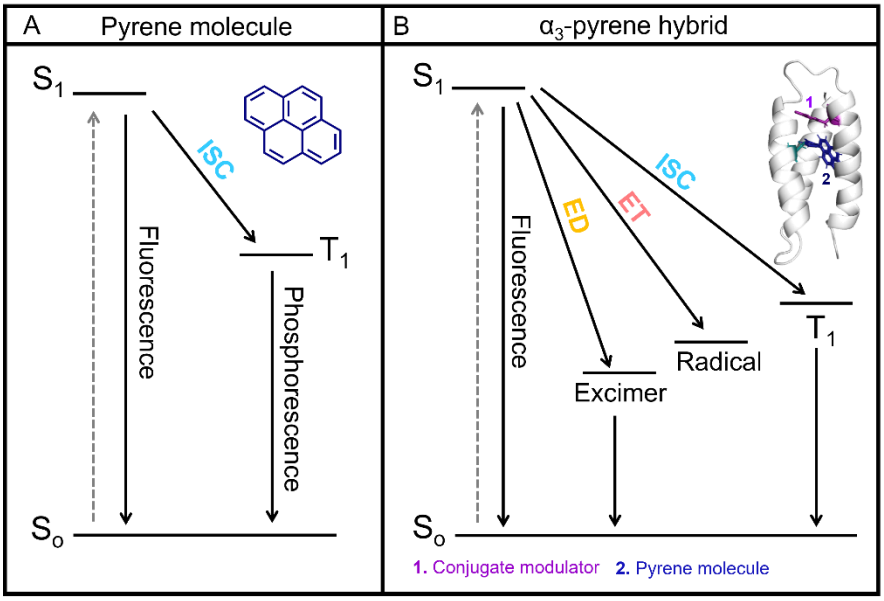

Figure 1. Left, pyrene S_1 excited-state decay scheme via fluorescence and intersystem crossing (ISC) promoted phosphorescence. Right, pyrene S1 excited state decay scheme embeeded in the protein via fluorescence, excimer emission, phosophorescence and radical recombination promoted by exciton dimerization (ED), intersystem crossing and electron transfer reactions inside the protein.

Anoxygenic Photosynthetic Bacteria-based Biocathodes for Green Hydrogen Production

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Photosynthetic purple non-sulfur bacteria (PNSB) have a particularly versatile metabolism that has inspired their implementation in a wide range of biohybrid electrochemical systems,[1] which can be applied for several purpose (i.e. biosensing,[2] electrochemical wastewater remediation,[3] energy generation, and the production of chemical commodities).[4] Various artificial approaches were utilized to facilitate the extracellular photo-induced electron transfer, however, the cathodic process has been consistently less explored compared to the anodic counterpart.[5] In this work, we report a sustainable approach to obtain a biohybrid photocathode for green hydrogen production using a home-made carbon based electrode modified with polydopamine. Specifically, a blend of poly-hydroxybutyrate (PHB) and carbon nanofibers (CF) were utilized to obtain a biocompatible electrode, which was modified by means of a redox-adhesive polydopamine-PNSB matrix.[6] The biohybrid system has been characterized by means of cyclic voltammetry and chronoamperometry, revealing the role of PNSB in the obtained cathodic photocurrent. The developed system was compared with biohybrid electrodes obtained utilizing commercial glassy carbon as support, showing a 30-35 fold enhanced photocurrent production. The possible future application of such system for green hydrogen production utilizing wastewater as substrate will be discussed in view of preliminary experiments performed utilizing various substrates.

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The role of eDNA in extracellular electron transport.

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Bioelectronic devices in which bio lms of photosynthetic bacteria are grown onto electrodes and used to harvest solar energy represent a highly promising and rapidly developing eld of renewable energy technology[1] However, these devices are currently limited by slow electron transfer between the bio lm and electrode surface; the mechanisms of electron transport through the extracellular matrix (ECM) that separates the two remain poorly understood.[2] It has been recently been suggested that extracellular DNA (eDNA) present in bio lms of Pseudomonas aeruginosa may play a role in extracellular electron transport (EET), possibly via the binding of redox-active mediator molecules such as pyocyanin[3] (Figure 1). We have used a combination of surface-study techniques such as neutron re ectometry and quartz crystal microbalance in combination with (photo)electrochemistry to better understand the role of DNA both as a conductive nanowire in the ECM, and in its interactions with such redox molecules. We compare both simple DNA oligomers and the more complex G4-quadruplex structures, that we hypothesise may have augmented electron transport abilities.

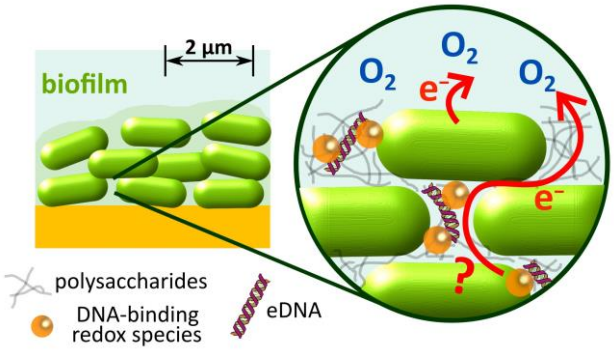

Figure 1: Schematic showing possible mechanisms for extracellular DNA (eDNA) facilitating electron transport through the extracellular matrix at the bio lm/electrode interface.

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Simulations of the Nucleosomal DNA : Mapping the Radical Cation Guanine

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An overexposure of DNA to an oxidative stress may results to various diseases related to mutations, especially cancers[1]. Therefore, understanding the behavior of the DNA oxidative damages is of medical and pharmaceutical interest. Guanine has the lowest ionization potential among the 4 DNA nucleobases[2] thus it is the most likely to be oxidized. However, the guanine redox properties can be impacted by the biomolecular environment around the nucleobases, which modulates the charge transfer ability of the DNA double strand. Then, the specific environment of the nucleosome, the fundamental unit of the chromatin, must be considered to understand DNA oxidative damages in cell. The nucleosome is composed of double-stranded DNA wrapped around four histone dimers rich in positively charged residues, which leads to a wide landscape of DNA-protein interaction.

Here, we investigate the factors and effects that can modulate the ionization potential of guanines in the nucleosome with a focus on their interactions with histone flexible N-terminal tails by means of a multiscale approach. We combine classical molecular dynamics simulation at the µs scale and QM/MM calculations based on the FO-DFTB/MM[3,4] approach. This method allows us to determine the ionization potential of a large number of nucleobases and the electronic coupling between them.

According to our results, the proximity of positive charges from histone tails or sodium cations seems to be the most predominant cause of variation of the ionization potential. Sequence and orientation towards the nucleosome core have little impact on this redox property. The electronic coupling values mostly depend on the geometry of the considered guanine pair, but the strongest ones are compatible with sub-nanosecond charge transfers (tens of meV). Our understanding of the combinatorial impacts of the structure and dynamics of nucleosome on the DNA charge transfer parameter is currently limited by our sampling but also by our use of conventional approach. Consequently, we want develop machine learning algorithms to improve our analysis and predictive power of nucleosomal guanine redox properties.

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Parallel symposia

Wednesday August 30

Afternoon

Brand new delivery approaches for PDT

Invited speakers:

IL76 Anzhela Galstyan (Duisburg-Essen, Germany)

Tracking light-triggered phototoxicity in photoactive nanomaterials

IL77 Girgis Obaid (Dallas, USA)

Nanolipid platforms: enablers of photodynamic tumor manipulation

IL78 Fabienne Dumoulin (Istanbul, Turkey)

Delivery strategies of photosensitizing phthalocyanines to tumours

IL79 Tayyaba Hasan (Boston, USA)

Photon is not only an actuator but an integral part of the delivery system

Oral communications:

OC86 Tiffany Ho: Intracellular insights into a novel EDTA-mediated nanoparticle delivery strategy for enhanced porphyrin delivery and photodynamic therapy

OC87 Davide Orsi: Nanostructures combining scintillating CeF3 and photoactive ZnO for X-ray Photodynamic Therapy of deep tumours

OC88 Nazar Vasyliv: New outlook on photodynamic therapy for glioblastoma cell lines.

OC89 Dong Wang: Peptide-Targeted Systems for Photodynamic Therapy

Tracking light-triggered phototoxicity in photoactive nanomaterials

Anzhela Galstyan [1]

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Self-assembly is a very efficient approach for the development of a variety of advanced functional materials with nano- or microscopic structures based on non-covalent interactions between molecules.[1] Although the mechanism of photogenerated ROS in such systems is well studied, there are very limited studies investigating the influence of intricate environmental factors, including those occurring in the cellular environment, on the self-assembly and thus the activity of the system. Synthesis and selfassembly properties of structurally closely related low-symmetry phthalocyanine derivatives will be presented showing how the lifetime criterion can help elucidate the mechanism of action to become a useful tool for quantitative analysis.

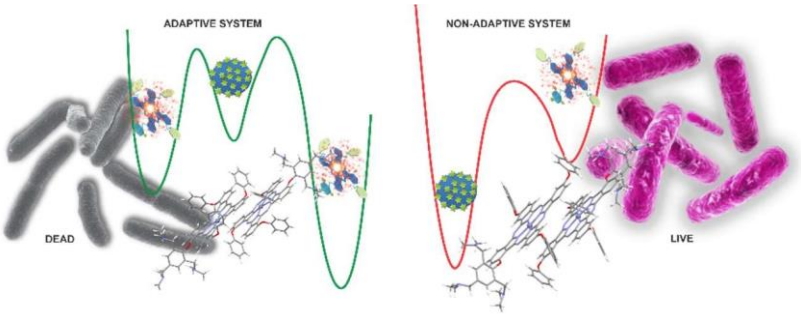

Figure. Illustration of adaptive and non-adaptive systems for aPDT.

The combination of sunlight and a reusable photocatalytic material capable of generating ROS from dissolved molecular oxygen is a promising concept for the development of sustainable systems for the disinfection and decontamination of natural or industrial waters. The design and synthesis of photoactive compounds that can be used as structural components of nanoscale materials and the structure-activity relationship of such systems will also be presented [2].

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Nanolipid platforms: enablers of photodynamic tumor manipulation

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Nanolipid platforms were the first nanoscale photosensitizer agents to be approved for photodynamic therapy (PDT).[1] Due to their versatility, various iterations have since been developed, which integrate multiple functionalities. These include multi-modal image guidance and combination therapies. Our work has focused on fine-tuning an array of nanolipid platforms as enablers of photodynamic tumor manipulation at multiple length scales: 1) manipulation at the nanoscale to reroute photosensitizers to different organelles,[2–4] 2) multi-receptor targeting of heterogeneity,[5–7] 3) stromal remodeling to promote chemotherapy responses[8,9], 4) improving tumor delivery and penetration, and 5) manipulation of the tumor immune landscape. An example of how PDT using nanolipid platforms can promote tumor delivery and penetration is presented in Figure 1. This work will be discussed in the context of pancreatic cancer and head and neck cancer, and how our strategies are enabling less toxic and more efficacious combination regimens.

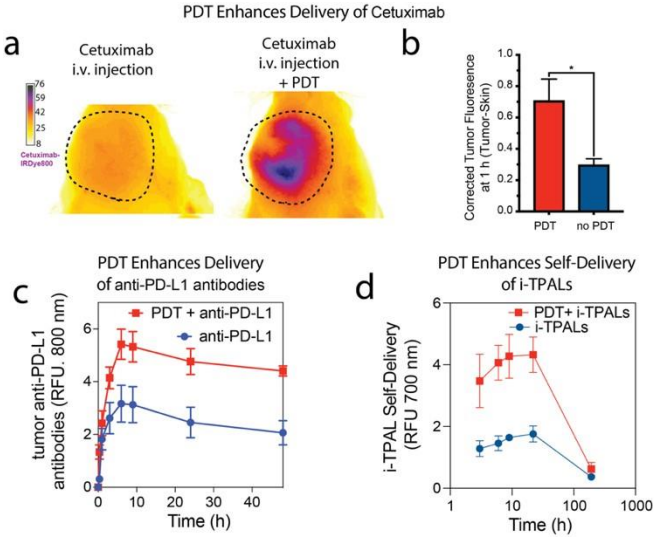

Fig. 1. PDT using nanolipid formulations of benzoporphyrin derivative improves bulk tumor delivery of Cetuximab in orthotopic FaDu HNSCC tumors (a) and anti-PD-L1 antibodies in orthotopic syngeneic AT-84 HNSCC tumors (b). PDT using a single, integrated and targeted photoactivable liposome (i-TPAL) construct enhances self-delivery in solid pancreatic tumors by 2.4-fold (d).

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Delivery strategies of photosensitizing phthalocyanines to tumours

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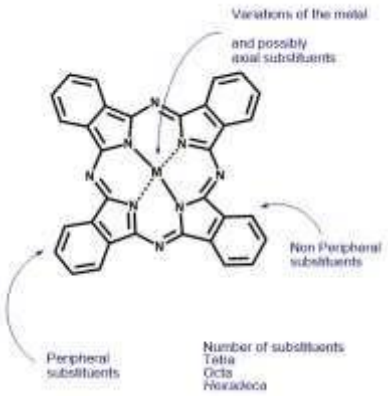
Phthalocyanines have an intense absorption at far-red wavelengths, which is extremely advantageous for PDT [1]. Taking phthalocyanines to tumours for anti-cancer PDT request either to make water-soluble derivatives [2] or to use delivery techniques. We have designed fluorinated phthalocyanines [3] for specific integration into liposomes [4] and other carriers such as PVP [5], mono-functionalized phthalocyanines for grafting onto bio-compatible polymers [6], and we have prepared phthalocyanine-based silesquioxane nanoparticles out of propargyl phthalocyanines [7]. The respective advantages and drawbacks of these tumours' delivery strategies will be discussed.



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Photon is not only an actuator but an integral part of the delivery system.

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There is an increasing understanding of disease mechanisms and their regulation. Simultaneously there is general acceptance that disabling single pathways, when they are believed to be the dominating pathway or targets, will not provide effective longterm cures for disease. Inhibition of a given pathway leads the cancer cell or the pathogen to commandeer compensatory mechanisms for its survival resulting in drug resistance. Combination of therapeutics addressing multiple mechanisms has therefore emerged as a desirable mode of disease management, but in that case pharmacokinetics and dosing now become complex and crucial. While the concept of combination treatment is by no means new, there are some newer emerging approaches to administering combination therapeutics that are exciting. These include the use of nanotechnology and light as a switch, which can provide cytotoxicity while at the same time priming the microenvironment and helping deliver molecules at the right time to the right place. Results from the literature and our own studies in the context of the potential of PDT-inspired combination therapeutics for cancer and microbiology using nanotechnology will be presented.

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Intracellular insights into a novel EDTA-mediated nanoparticle delivery strategy for enhanced porphyrin delivery and photodynamic therapy

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Porphysomes (PS) are liposome-like lipid nanoparticles engineered for porphyrin delivery, comprising >80,000 porphyrin-lipids per particle which enable their application for photodynamic therapy (PDT) and imaging[1]. One of the vital factors that can affect the extent of PDT efficacy is the effective intracellular delivery of photosensitizers in cancer cells. Recently, we have designed a new generation of PS nanoparticles (next-generation porphysomes; NPS), which utilize an EDTA-lipid mediated strategy to increase the uptake of porphyrin nanoparticles in cancer cells by 25-fold and improve PDT efficacy compared to the original PS platform[2]. Herein, we report the characterization of a new formulation of NPS, which possessed high fluorescence quenching (>99%) and stability in serum (>85% quenching efficiency) and at various pH conditions (pH 5.0-7.0; >98% quenching efficiency) over a period of 24 hours. We also demonstrate the ability of EDTA-lipids in this NPS nanoparticle structure for improving the cellular uptake of NPS by fluidizing the cell membrane in a detergent-like manner. NPS was also found to partially colocalize with endosomes in KB cervical cancer cells, whereas PS fluorescence signal was found dispersed throughout the cytosol. After a 6-hour KB cell incubation with NPS, significant photocytotoxicity (>50% cell killing) was observed after irradiation (photon density of 1.66 x 10^{19} *hv*/cm^2) with a 660 nm BioTable light box compared to the original PS (<5% cell killing). This work aims to identify the intracellular factors and mechanism involved in the enhanced PDT efficacy of NPS. Overall, the NPS nanoplatform encourages the adoption of this EDTA-mediated nanoparticle strategy to improve photosensitizer delivery and enhance phototherapeutic efficacy.

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Nanostructures combining scintillating CeF_3 and photoactive ZnO for X-ray Photodynamic Therapy of deep tumours

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X-ray Photodynamic Therapy (XPDT) is a nanostructure-based evolution of standard PDT, whose applicability to deep tumors is limited by the penetration depth of NIR/visible light. XPDT uses nanostructures that under Radiotherapy (RT) irradiation generate Reactive Oxygen Species (ROS) and singlet oxygen ($^1O_2$) [1]; the resulting oxidative stress reduces the viability of cancer cells, thus enhancing the efficacy of RT.

A XPDT nanostructure combine scintillating nanostructures and photosensitizers [2,3]. The scintillating part is excited by X-ray photons and by secondary particles produced as the beam penetrates the tissue; it then transfers energy to the photosensitizers, which in turn generate ROS and $^1O_2$. We present here nanostructures that combine scintillating CeF_3 nanoparticles and photoactive ZnO [2,4] following different synthesis strategies; the morphology, composition and optical properties of the resulting nanostructures have been characterized by Dynamic Light Scattering, SEM-EDX, SEM-Cathodoluminescence, TEM, Fluorescence and UV-Vis spectroscopies.

Direct detection of $^1O_2$ generated under X-ray irradiation was performed by measuring its feeble fluorescence emission at 1270nm, using a portable NIR fluorimeter based on a modern InGaAs SPAD detector coupled to a custom-made integrating sphere (Fig. 1) [5]. This novel experimental approach allowed us to determine the amount of generated $^1O_2$ per unit RT dose and unit nanostructure concentration, paving the way for further testing on cell cultures and animal models.

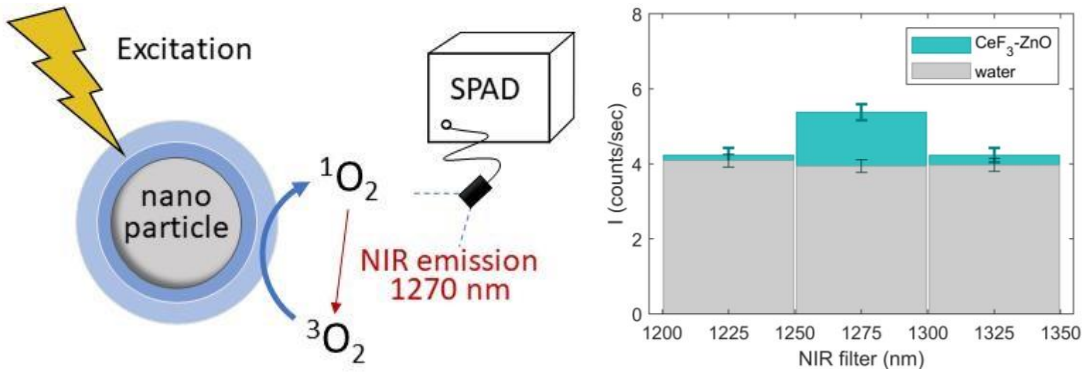

Figure 1. left) Direct detection of singlet oxygen generated by an XPDT nanostructure using NIR fluorimetry. right) $^1O_2$ NIR fluorescence signal measured in presence of CeF_3-ZnO XPDT nanostructures irradiated by 6MeV photons (cyan) compared to pure water (gray).

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New outlook on photodynamic therapy for glioblastoma cell lines.

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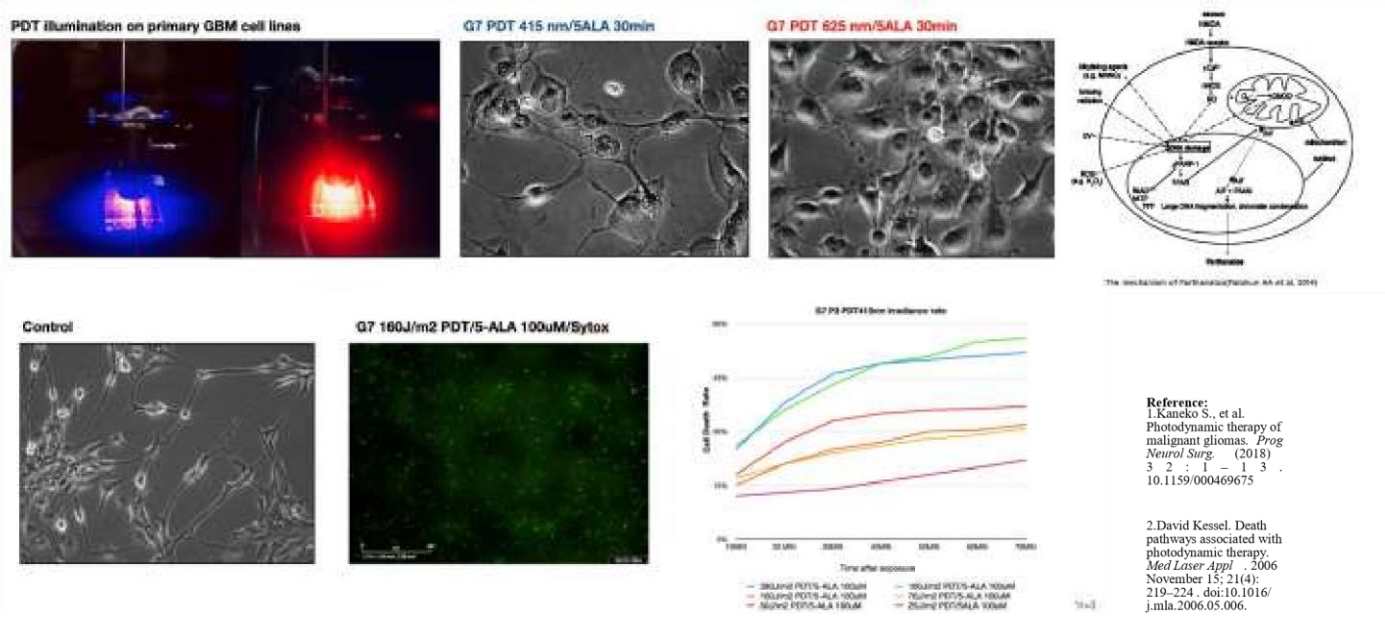
Although total gross resection of glioblastoma (GBM) followed by chemotherapy and fractioned radiotherapy prolongs the overall survival of patients with malignant glioma, medical treatment does not result in a complete recovery from the disease(1). Photodynamic therapy is not a novel method that has been utilised in neurosurgery because it affects tumour cells by causing cell death. However, the molecular mechanisms underlying glioma cell death remain unclear(2). This study aimed to elucidate the mechanism(s) by which PDT/5-ALA induces cell death in GBM cell lines. Live-cell imaging using IncuSyte Software detected significant cell death in the primary G7 glioblastoma cell line by adding Sytox Green. 55% dead G7 primary GBM cells were detected 1h after exposure to the irradiance of 160 J/m^2 PDT 415 nm/5-ALA100uM. The cell death rate increased proportionally to the irradiance rate, ranging from 25 J/m^2 to 390 J/m^2. Western blotting experiments revealed a caspase-independent cell death pathway with PARP1 cleavage. Therefore, the developed PARP inhibitors and BH-3 mimetics in combination with PDT/5-ALA are novel and promising tools in the standard of care for glioblastoma patients which may prolong the overall survival rate.



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Peptide-Targeted Systems for Photodynamic Therapy

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Photodynamic therapy (PDT) is a minimally invasive approach for the treatment of cancer and various other human disorders, based on the selective activation of photosensitizers (PSs) with light. At present, one of the most promising strategies for PDT and also fluorescence photodiagnosis (PDD) is to use 5aminolevulinic acid (ALA) as a prodrug to increase intracellular levels of the endogenous PS, protoporphyrin IX (PpIX). Although ALA-PDT has been shown to be a very promising clinical approach, the physicochemical properties and chemical reactivity of ALA present some challenges. These may be addressed by incorporation of ALA units into a variety of prodrug systems,[1] and we have previously shown that peptidebased prodrugs are an attractive way to improve the delivery of ALA, leading to enhanced PpIX accumulation and PDT effects.[2] In this study, we present a novel and easy approach to assemble prodrug systems to enhance the delivery of ALA to specific cell types by targeting with tumour-homing peptides. Our approach is based on a molecular core to which multiple ALA units (ALA dendron derivatives) are attached as the effector units, and with ALA itself connected by an ester bond. The core structure is also linked to a targeting peptide that is prepared by solid phase synthesis, with selective peptide attachment to the core being achieved via Cu-catalysed click chemistry. This combines the concept of ALA dendrimers and ALA-peptide prodrugs.[3] As proof of concept of this particular approach, we have prepared systems containing a bombesin-derived peptide that allows selective targeting of the GRP receptor (GRPR) which is overexpressed in a variety of tumours. A new generation of peptide- targeted ALA dendritic prodrugs was successfully synthesized, and the structure was optimized with respect to the peptide attachment and dendron units to obtain second-generation prodrugs. Targeted ALA delivery and

PpIX production with these prodrugs in GRPR-expressing PC3 cells have been investigated by fluorescence spectroscopy and confocal microscopy, and red and blue light-activated cell killings were evaluated using cell viability (MTT) assays.

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Impact of UV radiation in Human inflammatory health disorders

Invited speakers:

IL80 Kirsty Rutter (Manchester, UK)

UVR-induced skin inflammatory events in solar urticaria

IL81 Peter Wolf (Graz, Austria)

Microbiome modulation of UVR impact on skin

IL82 Richard Weller (Edinburgh, UK)

Sunlight and all-cause mortality

IL83 Prue Hart (Perth, Australia)

What we have learnt about UVB-induced immunomodulation from the PhoCIS trial

Oral communications:

OC90 Gareth Hazell: Upregulation of nitric oxide in vitro after low-dose artificial and natural sunlight exposure

OC91 Sarah Jelleschitz: Adduction of senescence associated aldehydophospholipids to a collagen matrix affects the responses of residing immune cells

OC92 Marina Venturini: Field validation of a satellite-based dosimeter of personal solar exposure

UVR-induced skin inflammatory events in solar urticarial

Kirsty J. Rutter[1,2], Michael Peake[1], Nathan J. Hawkshaw[1], Rachel Scholey[3], Silvia Bulfone-Paus[1], Peter S. Friedmann[4], *Mark D. Farrar[1,2], *Lesley E. Rhodes[1,2]

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Solar urticaria (SU) is a rare inducible chronic urticaria characterised by rapid onset of urticarial signs and symptoms on exposure to ultraviolet and/or visible radiation. Symptoms can be extremely disabling, with high negative impact on quality of life. It has been assumed that IgE-mediated mast cell degranulation drives the disease, but the evolution and resolution of the SU response and the immune cells, molecules and mechanisms involved are not well understood.

We investigated cutaneous molecular and cellular responses to solar simulated UVR (SSR) in patients with SU, with comparison to healthy controls (HC), using immunofluorescence and transcriptomic techniques. Patients with diagnosed SU and HC participants were exposed to a low (physiological) dose of SSR to photoprotected upper buttock skin. A series of biopsies were taken from adjacent unexposed skin and at 30 minutes, 3 hours and 24 hours after SSR exposure. Biopsies were assessed by immunohistochemistry and bulk RNA-sequencing analysis.

Urticarial responses in SU were accompanied by enrichment of immune pathways relating to innate inflammatory cell recruitment and activation, notably involving neutrophils and eosinophils, which was not observed in HC. Pro-inflammatory cytokine and chemokine genes were upregulated (including *IL20, IL6* and *CXCL8*) or identified as upstream regulators (including TNF, IL1β and IFNγ) in SU but not HC. Mast cell numbers did not change following SSR exposure, but free dermal tryptase was observed in SU. IgE, FcεR1 and STAT3 were identified as upstream regulators following SSR exposure, and there was an increase in the number of mast cells expressing phosphorylated STAT3, suggesting a mechanism of mast cell activation in SU. Resolution of clinical features in SU by 24 hours was accompanied by resolution of inflammatory gene signature profiles.

Greater understanding of pathophysiological processes in SU highlighted by this first transcriptomic study of SU may help to suggest potential therapeutic targets.

Microbiome modulation of UVR impact on skin

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The human body hosts innumerable microorganisms on the skin, together named the cutaneous microbiome, maintaining homeostasis with the immune system. Ultraviolet radiation (UVR) hits first the microbiome on the surface of the human body before it reaches any cell of the human organism. Thus, it comes with no surprise that UVR with its bactericidal effects may affect homeostasis in health and disease. However, UVR may also have indirect effects on the microbiome of the skin and beyond (by induction of antimicrobial peptides (AMPs), cis-urocanic acid (UCA), and vitamin D, and other molecules). For instance, UV-induced cis-UCA has been shown to affect the growth and diversity of microbiome. Notably, certain bacteria metabolize (cis-)UCA and thus their elimination may affect the immune response to exposure to UVR. Furthermore, UVR can also directly induce the production and release of AMPs by microorganisms or human cells, interfering with the response to its exposure. Experimental studies with germ-free or disinfected wildtype mice have indicated that an intact microbiome (of the skin) has immune protective properties. Together, a healthy microbiome of the skin may help protecting from UV carcinogenesis. On the other hand, the effects of UVR on the skin in diseases such as atopic dermatitis and cutaneous T cell lymphoma may be beneficial (by diminishing the growth of certain bacteria such as *Staphylococcus aureus*) in restoring homeostasis, and in turn reducing inflammation in diseased skin.

Sunlight and all-cause mortality

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Excess ultraviolet radiation is a risk factor for skin cancers in white skinned populations. Several UV driven beneficial mechanisms for human health have been identified. These include, vitamin D synthesis, nitric oxide release and effects on immune homeostasis. Skin colour modulates biological responses to UV.

All-cause mortality is a ready indicator of the balance between beneficial and deleterious effects of an environmental exposure. Data from several groups in Sweden correlate increased sun exposure with reduced all-cause mortality. We have examined the effects of UV exposure on population health using the UK Biobank, a large prospective cohort study in the UK. 376,279 participants, with Fitzpatrick 1-3 skin on whom complete data was available were studied. Median follow-up time was 12.7 years. Two measures of UV exposure were identified. Sun seeking behavior is associated with sun bed users. 4.87% of the cohort who used sunbeds were identified as sun-seekers. Higher latitude correlates with reduced UV exposure and a northerliness variable was used to mark UV exposure in the second analysis. Corrections were made for the confounders: age, gender, BMI, Deprivation, smoking, exercise, employment, education. Serum Vitamin D is a biomarker for UV and correlated linearly with latitude and sun-seeking behavior: 67.2 (66.9, 67.5)in sun-seekers and 48.9 (48.9, 49.0) in non-sun-seekers.

All-cause (HR 0.86, 0.8-0.93), Cardiovascular (0.81, 0.68-0.95), Cancer (0.86, 0.77-0.95) and non cancer/non-CVD (0.86, 0.77-0.95) mortality were all reduced in sun-seekers. For each 300km southerly increment of home domicile, all-cause (HR 0.94, 0.92-0.96), Cardiovascular (0.91, 0.86-0.95), Cancer (0.93, 0.9-0.96) and non-cancer/non CVD (0.97, 0.92-1.01) mortality were reduced. This study confirms Scandinavian data. For high latitude European populations, the benefits of sunlight exposure outweigh the risks

Reference : Insufficient Sun Exposure Has Become a Real Public Health Problem doi.org/10.3390/ijerph17145014

What we have learnt about UVB-induced immunomodulation from the PhoCIS trial

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In the PhoCIS trial (Phototherapy for Clinically Isolated Syndrome), participants with CIS, the earliest form of multiple sclerosis (MS), were randomised into a treatment arm with exposure to suberythemal narrowband UVB (311 nm) to the full body 3 times per week for 8 weeks, or to standard care. This phototherapy protocol is used frequently for patients with psoriasis. All participants were followed clinically and immunologically for 12 months. After 12 months, all the control participants had converted from CIS to MS. In contrast, 70% of those who received phototherapy had converted to MS, with 30% showing no new lesions after 12 months on magnetic resonance imaging.

We have been using a biobank of frozen cells and sera from the participants to investigate the mechanisms by which narrowband UVB may alter immune cell networks. This may give clues to the mechanism by which narrowband UVB may be immunomodulatory and alter the course of MS progression. Alternatively, biomarkers associated with exposure of skin to narrowband UVB may be uncovered. There was a significant decreased prevalence of switched memory B cells, and a complementary increase in prevalence of naïve B cells after 2 and 3 months in those receiving phototherapy, compared with those not receiving phototherapy (Trend et al., Sci Rep 2019). There was also a decreased ability after 2 months of blood B cells to produce the inflammatory mediator TNF upon polyclonal activation (Trend et al., Clin Trans Immunol 2020). This result suggested a priming or epigenetic effect of UV exposure.

To identify products from UVB-irradiated skin that may be immunologically active and aid in the communication between UVB-irradiated skin and internal tissues, both the proteome and the metabolome have been analysed in sera taken longitudinally from the participants receiving narrowband UVB, compared with the control CIS participants. Ninety-two (92) proteins were assessed by OLINK^{R} and 1353 analytes by General Metabolomics^{R}. After 1 week, 1 month, 2 months, 3 months, 6 months and 12 months, there were 24, 12, 3, 22, 18 and 5 significant proteomic changes from baseline (time 0) in the sera from those receiving narrowband UVB phototherapy. In contrast, there were no significant proteomic changes from baseline (time 0) in the sera from the control CIS participants over the 12 months of the trial. Changes were predominantly a decrease in analytes associated with inflammation (CASP8, SIRT2, 4EBP1, CXCL6, CXCL11, ADA). After 1 month, there were significant metabolome changes in those receiving phototherapy with no significant changes detected in the serum from the control CIS participants. Significant changes reflected altered tryptophan metabolism and lysine degradation. Many of these changes (decreased 4EBP1, SIRT2, CASP8) persisted for 6 months after UVB therapy.

In conclusion, suberythemal narrowband UVB delivered to the skin caused significant changes to the cells, proteins and analytes in the blood, many lasting months. We propose these changes may be important to an improved understanding of cell-cell communication and immunological networks associated with a disease such as MS.

Upregulation of nitric oxide in vitro after low-dose artificial and natural sunlight exposure

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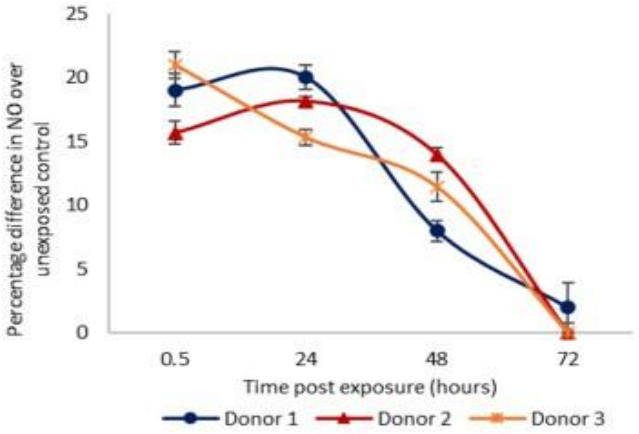
Hypertension is a chronic elevation of blood pressure, implicated in the risk of future cardiovascular events. Nitric oxide (NO) is an endogenous molecule that acts to reduce blood pressure through a clear vasodilatory effect, with production diminishing as we age (Shannon et al, 2022). This not only contributes to the rise in hypertension among the elderly but may potentially drive other disorders. Mitigation of hypertension, for example by an upregulation of NO, could have an enormous impact on health and personal wellbeing.

It has been shown that NO may be produced via the breakdown of metabolites such as nitrates or nitrites held at high yields within the skin by artificial sunlight (Holliman et al, 2017). In our work, we have verified this effect using primary *in vitro* skin cell lines demonstrating not only that within all skin cells nitrite is readily broken down by low level ultraviolet-A (UV-A) irradiation, but that this effect persists for up to at least 48 hours after exposure (Hazell et al, 2022). We highlight that this secondary upregulation in nitric oxide via UV-A is biphasic, occurring not only from salts within the skin but via complex cross-talk with upregulation of calcium dependent nitric oxide synthase enzyme isoforms (eNOS and nNOS) and to a lesser extent non-calcium driven iNOS at low dose.

Further, we utilise natural UK summers day sunlight exposure on *in vitro* skin cells to assess this response against direct DNA damage. Here we highlight that sunlight exposure as low as 1-2 SED can readily induce NO upregulation from dermal skin cells with minimal negative effect. These results highlight that there is a potential cardioprotective effect from low-dose sunlight exposure, while avoiding damage to the skin. Further development in this area could hold beneficial outcomes with regards to hypertension and cardiovascular function especially for the elderly population



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Adduction of senescence associated aldehydophospholipids to a collagen matrix affects the responses of residing immune cells

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Previously, we found specific chemically reactive lipids that accumulate in oxidatively stressed dermal fibroblasts.[1,2] In UV exposed but also prematurely senescent fibroblasts we had identified *1-palmitoyl-2-(5'-oxo-valeroyl)-sn-glycero-3-phosphocholine (POVPC) and 1-palmitoyl-2-(9'-oxononanoyl)-sn-glycero-3-phosphocholine (PONPC)* and lipids present in oxidized 1-palmitoyl-2arachidonyl-sn-glycero-3-phosphorylcholine (OxPAPC). Some of these lipids contain a carbonyl group which have the potential to bind covalently to proteins. Collagen adducts of 4Hydroxynonenal (4-HNE), PONPC and other aldehydic compounds have been identified using

HPLC-Mass Spectrometry. We studied if these lipids can modify the skin's extracellular matrix (ECM) proteins and investigated whether this would affect the function of cells residing within this modified matrix. We identified that collagens I, II and IV were prone to adduct formation with reactive lipids. Bone marrow derived monocytes were cultured on a matrix modified with lipids and gene expression changes induced by matrix modification have been investigated. The cells showed an early senescent phenotype (reduced LaminB1 and increased p21 expression) as well as low grade synthesis of IL1a and IL8 chemokines. After an additional stimulus with LPS cells on an unmodified matrix showed a transcriptomic response in a pattern specific for the type of aldehydolipid modification whereas cells cultured on 4-HNE modified matrix showed broad inhibition of LPS induced inflammatory genes. The gene expression of cells grown on the aldehydophoshpolipids matrix induced TNFα expression. These findings could contribute to explain how senescent cell derived factors can modulate inflammation and facilitate the evasion of clearance by phagocytic cells.

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Field validation of a satellite-based dosimeter of personal solar exposure

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The results of a field study validating a novel satellite-based digital solution for personal solar exposure dosimetry ("ExpoDose" by siHealth Ltd, UK) will be presented. A mobile app tracking the user's position and satellite data processing are used for providing up-to-date personal solar dosimetry data for any action spectrum (e.g., erythema, vitamin D synthesis, DNA damage) and different body sites via a dedicated webportal. The app is autonomous and requires no external sensors neither direct exposure of the smartphone to sunlight since solar radiation is monitored from satellite (i.e. smartphone can be kept in a pocket).

This digital solution is enabled by the HappySun® satellite-based technology for near real-time monitoring of solar irradiance worldwide (already scientifically validated in multiple studies [1, 2, 3, 4]) and by an automatic outdoor position detection technology based on AI models applied to smartphone sensors data. These two technologies combined allow the remote monitoring of solar dose with 1 minute resolution for any number of users, multiple action spectra and multiple body sites simultaneously.

Data collected with the ExpoDose system were compared to 10 high-quality wearable UV electronic dosimeters

(Scienterra Ltd, New Zealand) measuring irradiance on 10 measurement planes oriented over a range of 4 different zenith angles and 8 compass points. The wearable dosimeters were calibrated with a research standard UV-erythemal radiometer (Kipp & Zonen, Netherlands) installed horizontally. Data were collected during spring and summer in Harwell Campus (Oxfordshire, UK) and in Brescia (Italy).

Preliminary results show a high accuracy of the satellite-based solar dosimetry system, yielding an R^2 correlation coefficient of 0.90 and a mean absolute error (MAE) of 21% on the horizontal plane. Moreover, the automatic outdoor detection component has been tested in a broad range of scenarios on smartphones running both Android and iOS operating systems. Using cross-validation techniques over multiple smartphone models, detection accuracies resulted over 92% on Android and 84% on iOS.

A remote personal solar monitoring system has great promise for use in multi-participatory studies that need to account for personal solar exposure levels of study subjects. Carefully calibrated and maintained high-quality solar dosimeters have been demonstrated to have an estimated error of 12% [5]. Compared to remote personal dosimetry, they are costly, require expertise to maintain and calibrate and require high levels of attention and compliancy from experiment subjects.

So, the ExpoDose system can be effectively used for research and clinical studies, replacing the need for costly and time-consuming physical dosimeters in multi-participatory longitudinal solar exposure studies.

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Photoperception and acclimation to changing light and CO_2 in microalgae

Invited speakers:

IL84 François-Yves Bouget (Banyuls-sur-Mer, France)

Photoperiodism in cosmopolitan green picophytoplankton

IL85 Dimitris Petroutsos (Uppsala, Sweden)

Blue light perception via PHOTOTROPIN controls photosynthetic carbon partitioning in green microalgae

IL86 Marianne Jaubert (Paris, France)

Underwater light sensing: activity of a diatom phytochrome photoreceptor in the Oceans

IL87 Emanuel Sanz-Luque (Cordoba, Spain)

Transcriptional regulation of Non-Photochemical Quenching in response to different light intensities and wavelengths in the microalga Chlamydomonas reinhardtii

Photoperiodism in cosmopolitan picophytoplankton

Francois-Yves Bouget[1], Louis Dennu[1], Cédric Mariac[2], Janaina Rigonato[3], Jean-Claude Lozano[1], Valérie Vergé[1], Angela Falciatore[4], Olivier Jaillon[3], Francois Sabot[2] and Martine Devic[1]

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Marine Phytoplankton accounts for half of the primary production of our planet, phytoplankton growth often occurring as seasonal blooms. By studying a 7-year time series on a coastal Mediterranean site (Banyuls Bay, France), we showed highly reproducible seasonal patterns of phytoplankton occurrence, particularly of abundant green picoeukaryotes (*Bathycoccu*s, *Micromonas*, and *Ostreococcus* genera) belonging to the class of Mamiellophyceae [1,2]. Metagenomics surveys in the World ocean revealed that the species *Bathycoccus prasinos* has a cosmopolitan distribution from poles to temperate regions in both northern and southern hemispheres. We isolated and genotyped *B. prasinos* strains from various geographic locations (arctic, temperate and austral Ocean) and during seasonal blooms in the Bay of Banyuls [3]. The genomes of 44 strains corresponding to the most diverse haplotypes were fully sequenced by Oxford Nanopore Technology and/or Illumina Sequencing. Several genetic variants associated to specific latitude or seasons display distinct responses to photoperiod/temperature in culture. The role of genetic structural variations and of the circadian clock in adaptation of phytoplankton to photoperiod (latitude and seasons) will be discussed.

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Blue light perception via PHOTOTROPIN controls photosynthetic carbon partitioning in green microalgae.

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While in leaf cells of higher plants sucrose and starch are the primary sinks for carbon fixed by photosynthesis, sucrose appears to be an insignificant metabolite in *Chlamydomonas* cells, where starch is the dominant carbon sink. Starch synthesis occurs during the day, using global outputs of photosynthesis and its degradation starts as night falls to sustain energy-demanding cellular functions. Light perception and starch metabolism have been associated in *Arabidopsis* but such a link has not been established in microalgae.

We have unveiled a complex PHOTOTROPIN-dependent signalling cascade, linking blue light perception with starch accumulation in *Chlamydomonas.* Briefly, PHOTOTROPIN (PHOT) represses GAP1 (glyceraldehyde-3-phosphate dehydrogenase) that acts as enhancer of starch accumulation. This PHOT-dependent regulation of starch metabolism takes place via the Ser/Thr protein kinase PMSK1 (Phototropin-Mediated Signaling Kinase). Serine 94 (S94) of PMSK1 is phosphorylated in the dark and gets de-phosphorylated upon exposure to blue-light in the Wild-Type (WT) while PMSK1 S94 remains phosphorylated at both dark and light conditions in the *phot* mutant. Intriguingly, overexpression of PMSK1^{S94D}, mimicking a phosphorylated S94, in WT cells leads to high *GAP1* and starch levels and conversely, overexpression of PMSK1^{S94A}, mimicking a dephosphorylated S94, in *phot* cells leads to low *GAP1* and starch levels. Our data strongly suggest that PMSK1 acts as a novel regulator that drives PHOT signaling pathways through post-translational modifications, controlling carbon metabolism. These findings have significant implications for our understanding of the intricate interplay between photoreceptor signaling and metabolism in photosynthetic organisms, and may potentially pave the way for the development of novel biotechnological applications.

Underwater light sensing : activity of a diatom phytochrome photoreceptor in the Oceans

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Marine microalgae are considered responsible for about half of the primary productivity on Earth. Hence, light plays an essential role as source of energy through photosynthesis for these unicellular organisms. But light is also an important environmental cue, accurately detected by photoreceptor proteins and triggering adjustment of cell physiology accordingly. In addition to the daily and seasonal variations of sunlight intensity, duration and spectrum, underwater light field is structured with depth, with a strong attenuation of long wavelengths such as red (R) and far-red (FR). Surprisingly, diatoms, predominant marine microalgae, have phytochromes photoreceptors, which are R/FR sensors in land plants. We have shown that diatom phytochromes (DPH) do exhibit R/FR absorption spectra and induce expression of a set of genes upon FR light exposure. This absorption spectrum appears widely shared among the DPH we have characterized from different diatom species. However, how a R/FR receptor is activated in the red-poor marine environment is a puzzle. By measuring the responses triggered by DPH photoreceptor *in vivo* thanks to a reporter system set in the model diatom species *Phaeodactylum tricornutum*, we showed that DPH activity is not solely dependent on the ratio of long R/FR wavebands, but extends to the entire visible light spectra. Modelized in an oceanic context, DPH activity changes with light fields that reflect depth variations and the presence of other photosynthetic organisms. This analysis revealing novel perspectives about phytochrome sensing abilities opens up new insights into the role of phytochrome-mediated light sensing in the oceans.

Transcriptional regulation of Non-Photochemical Quenching in response to different light intensities and wavelengths in the microalga *Chlamydomonas reinhardtii*

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Photosynthetic organisms have evolved in highly dynamic light environments, with variations in sunlight intensity and quality depending on the time of the day, atmospheric conditions, and canopy effect. These organisms have developed mechanisms to optimize the capture and utilization of light energy when the intensity is low and to quench excitation energy when absorbed light exceeds cellular demands for CO_2 fixation. In the latter condition, the first line of defense entails energy dissipation as heat within the photosynthetic antenna complexes (qE). In *Chlamydomonas*, three members of the light-harvesting protein family (LHCSR1, LHCSR3, and PSBS) have been ascribed to this function. High light intensities dramatically induce the expression of the genes encoding these proteins[1]. However, cells need to accumulate these genes before exposure to high light, anticipating the stress and avoiding the generation of reactive oxygen species. We have studied the RNA accumulation of *LHCSR1*, *LHCSR3,* and *PSBS1* in dark-to-light transitions at different light intensities and wavelengths. Our data indicate that very low light is enough to lead to a marked increase in the *LHCSRs* and *PSBS* transcript levels, and this LL-elicited accumulation is strongly dependent on blue light photo-perception by PHOT1 and only partially controlled by photosynthetic electron flow. Our results also show that the UV-B photoreceptor (UVR8) mediates the induction of these genes even in the absence of PAR light. Overall, our data shed light on the complex transcriptional regulation of the photoprotective genes, allowing *Chlamydomonas* cells to modulate the transcript levels and pre-acclimate to changing light conditions.

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Non-canonical DNA structures: from biological targets to (opto)electronic materials

Invited speakers:

IL88 Jean Louis Mergny (Palaiseau, France)

Nucleic acid quadruplexes: not just structural oddities?

IL89 Alexandr Kotlyar (Tel Aviv, Israel)

Silver Ions-Driven Folding of Polyguanine Strands into Double- and Quadruple-Helical Structures

IL90 Janez Plavec (Ljubljana, Slovenia)

NMR insights into 3D structure of G-rich DNA

IL91 Roberto Improta (Napoli, Italy)

Photophysics and photochemistry of DNA quadruple helices and I-motifs: insights from Quantum Mechanical calculations.

Oral communications:

OC93 Ilse Manet: Naphthalene diimides as versatile platforms for biomedical applications focusing on Gquadruplex

OC94 Carlos Montero: Lipid peroxidation and DNA damage: photoreactivity of cytosine-derived etheno adducts

OC95 Lara Martinez: Excited state processes in non-canonical DNA structures: insights from quantum mechanical calculations.

Nucleic acid quadruplexes: not just structural oddities?

Jean-Louis Mergny 1

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G-quadruplexes (“G4”) and i-DNA are unusual nucleic acid structures which can find applications in biology, medicine, as well as biotech- and nano-technologies. G4 can be formed intramolecularly by Grich DNA or RNA sequences. We are developping biophysical and biochemical tools to understand their folding and polymorphism, both *in vitro* and in cells, as well as the influence of external parameters such as pH, ionic conditions or crowding. Absorbance, Fluorescence and Circular Dichroism provide interesting information on these structures.

In parallel, we propose a new algorithm for the prediction of G4 propensity and apply it to the analysis of a number of organisms. We are interested in quadruplex-prone regions conserved in the genomes of a number of Archaea, pathogens such as bacteria, helminths, viruses (HIV, HCV, Ebola, SARS-CoV2...) as and in "G4- poor" model organism such as *Plasmodium falciparum* to confirm the importance of G-quadruplexes in biology. In addition, we are investigating new families of G4 ligands (G4L), either as fluorescent light-up probes or as anti-viral or anti-parasitic drugs.

Silver Ions-Driven Folding of Polyguanine Strands into Double- and Quadruple-Helical Structures

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Silver ions (Ag^+) are known to tightly and selectively bind to cytosine and guanine bases composing DNA. Binding of the ions to the bases of long (hundreds and thousands of nucleotides) poly(dG) at 1 to 2 (Ag to base) molar ratio leads to considerable changes in the circular dichroism (CD) spectrum of the molecule (Fig. 1A) indicating for conformational rearrangement of the DNA. Indeed, formation of circular structures, similar to those of DNA plasmids, was detected by AFM (Fig. 1B).

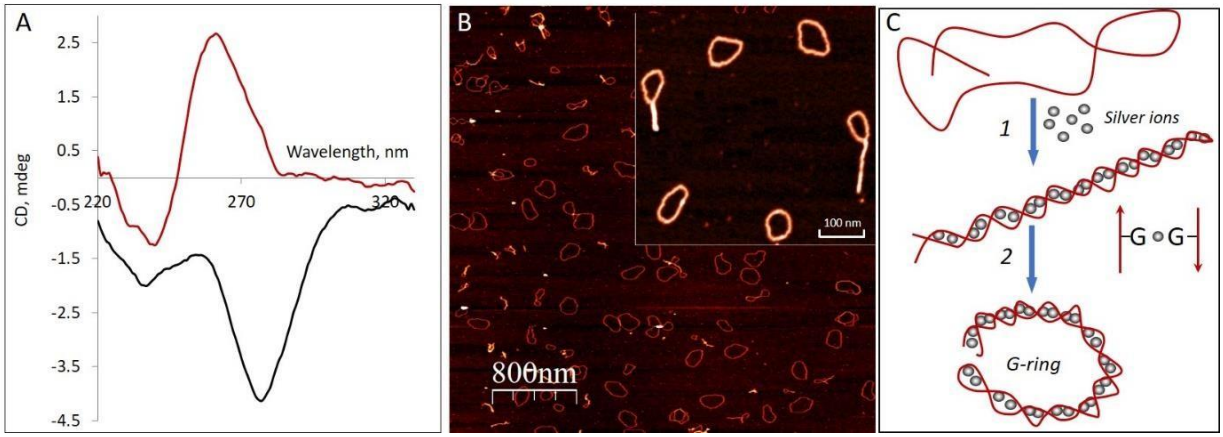

Figure 1. Folding of G-strand in the presence of silver ions. A- CD spectra of a G-strand (red curve) conjugated with silver ions at 2 to 1 base to ion ratio (black curve). B – AFM images and schematic drawings of structures formed during folding of a conjugate between 1400-base G-strand and silver ions (at 2 to 1 base to ion ratio). Inset shows ring-shaped molecule folding into a compact rod-shaped molecule. Two partly folded molecules (on the right and the left side of the image) are seen as stick-shaped structures with a loop at one of their ends. C – Scheme depicting conjugation of a G-strand (red curve) with silver ions (grey spheres) followed by folding of the conjugate into a circular ring-shaped structure, G-ring (reaction 2). The structure is stabilized by Ag^+-mediated G-G pairing.

The measured average contour length of the rings is about half that of the parent poly(dG)-poly(dC) molecules used for preparation of the homoguanidine strand suggesting that the strand is folded on itself such that the two halves are running in opposite directions. The halves are hybridized through Ag^+mediated G-G pairing to form a stable left-handed helix (Fig. 1C, reaction 1). The edges of the helix approach each other probably due to mechanical tension caused by the helical spin resulting in an opened ring-shaped structure (Fig.1C, reaction 2). Further twisting of this ring-shaped structure in a screw-like fashion resulted in a compact and stiff rod-shaped molecule (inset in Fig.1B).

The poly(dG)-Ag-based conjugates (both ring- and rod-shaped) are stiff and very stable under ambient and elevated temperatures (don't degrade at 90□C). High stability and the presence of redox active silver ions make these new conjugates promissing candidates nanobioelectronic devices and nanobiosensors.

NMR insights into 3D structure of G-rich DNA

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The typical double-stranded DNA conformation is the B-DNA form. However, certain sequences promote the formation of other structures under certain environmental conditions. During important cellular processes, DNA unwinds into a single-stranded form that can adopt multiple 3D structures that differ from the canonical Watson and Crick double helix. Secondary structures such as G-quadruplex and i-motifs are associated with many different biological functions of DNA. The best studied non-canonical DNA structures are G-quadruplexes. In recent years, experimental evidence has accumulated that these structures form *in vivo* and play essential biological roles, including regulation of gene expression, replication, and telomere maintenance.

Nuclear Magnetic Resonance, NMR, is a well-established technique for studying a wide range of topics related to the structure, dynamics, and function of biomolecules. Our laboratory uses NMR spectroscopy in combination with complementary methods to reveal structural details of four-stranded DNA architectures in terms of sequence details, presence of cosolutes and inorganic salts, pH, interaction with (heterocyclic) ligands, and folding pathways.[1-7] We have recently described a new family of tetrahelical structures that are distinctly different from G-quadruplexes, although they contain the Gquadruplex folding motif (i.e., d[(GGGNn)3GGG]). These sequences with Nn=AGCGA exhibit topologies characterized by a tetrahelical core of AGCGA repeats connected by edge-like loops of different lengths stabilized by G-G base pairs in N1-carbonyl geometry.

Different DNA structures open up a variety of targeting possibilities due to their local and dynamic properties. Due to their biological relevance, G-quadruplexes are attractive targets for drug development. Human telomeric G-quadruplex structures are attractive targets for anticancer drug development, but polymorphism of the targets complicates their development. Different ligands prefer different folds, and very few complexes have been solved at high resolution. Phen-DC3, bisquinoliniumderivatized phenanthroline dicarboxamide, one of the best-known G-quadruplex ligands characterized by high binding affinity and selectivity, causes dTAGGG(TTAGGG)$_3$ to completely change fold in KCl solution, with the ligand intercalating between two G-quartets.[7]

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Photophysics and photochemistry of DNA quadruple helices and I-motifs: insights from Quantum Mechanical calculations.

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In the last decade Quantum Mechanical calculations, together with Time-Resolved spectroscopy, allowed crucial advances in our understanding of the processes triggered in Nucleic Acids by Absorption of UV light,[13] which can have many pathological consequences (damage of the genetic code, apoptosis, carcinogenesis). [4] In our contribution, we concisely review the most significant results obtained in the study of Guanine-rich DNA sequences arranged in quadruple helices (G-Quadruplexes) [5-8], showing that the experimental steady state absorption and circular dichroism spectra of different topologies can be reproduced and assigned and that the most important decay pathways involve localization of the excitation over a single base or on two stacked guanines, excimers with different degrees of charge transfer character. We also report the first results obtained in the study of I-motifs, the peculiar 'intercalated' structure that can be adopted by NA sequences rich in cytosine, stabilized by emi-protonated $(CHC)^+$ pairs, i.e. two base-paired cytidines, 'sharing' one proton between the N3 atoms. Finally, we discuss some of the chemical physical effects ruling the photoactivated processes in NA, and the related methodological challenges, [9-11] which are common to many other multi-chromophore assemblies of biological and technological interest.[3]

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Naphthalene diimides as versatile platforms for biomedical applications focusing on G-quadruplex

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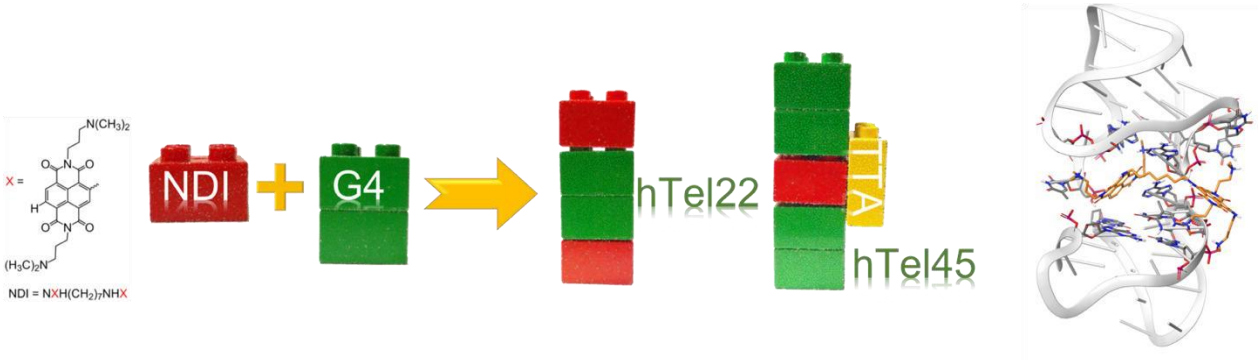
Naphthalene diimides (NDIs) are extremely versatile platforms explored for the development of different applications. Thanks to their chemical accessibility and large planar surface NDIs have been explored as appealing platform to design RNA and DNA G-quadruplex (G4) ligands that are able to interfere with metabolic pathways involving this non-canonical form of DNA/RNA. Additionally, their optoelectronic properties can be tuned by substituents on the aromatic core, thus giving origin to absorption and emission in the visible wavelength range, which makes them appealing also for fluorescence imaging and photodynamic therapy (PDT). We have published a series of NDI monomers, and NDI and NDI/core-extended NDI dyads having excellent water solubility merged with very promising features for biomedical applications.[1-2] The NDI optical properties were used to study their affinity for G4 and double stranded DNA. The NDI dyads display interesting fluorescence turn-on behavior upon recognition of the G4 target in solution.[2] We were able to identify the binding site of these dyads in multiple telomeric G4 by means of a in-depth fluorescence lifetime and circular dichroism study. The NDI fluorescence was further exploited to study cellular localization and interaction with G4-rich nucleoli by means of a confocal fluorescence lifetime imaging. These studies were completed with biological assays to determine the IC50 values of the monomers and dyads and to asses intracellular DNA damage. One monomeric tetra-substituted NDI compound induced photocytotoxicity.[2] Some of the NDI dyads have excellent IC50 values in the nM range on several tumor cell lines and are able to induce a DNA damage response at the telomeric level [2]. A dyad composed of one NDI unit and one extended NDI unit has higher IC50 values but its toxicity is markedly higher for tumor cells compared to healthy cell models making it a more suitable candidate for therapeutic applications. Overall, these prpmising features stimulate further research on the NDI versatile platform.



Cartoon of the interaction of a NDI dyad with telomeric DNA with 1 or 2 G4 units

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Lipid Peroxidation and DNA Damage: Photoreactivity of Cytosine-Derived Etheno Adducts

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Lipid peroxidation is a biochemical process that involves the oxidation of polyunsaturated fatty acids, the components of cell membranes. In this process, highly reactive species, such as α,β-unsaturated aldehydes, are generated and react with DNA bases, forming the so-called **etheno adducts**. These DNA lesions exhibit **mutagenic properties**[1]. Indeed, the increase of lipid peroxidation in inflammatory processes[2] has been related to the high levels of etheno adducts in diseases that can lead to cancer, such as Chron's disease or ulcerative colitis[1]. Consequently, they are excellent **bio-markers** for different types of cancer (lung, colon or prostate cancer). Although their mutagenic properties have been clearly established, their photoreactivity has not been studied yet.

Here, the attention is centered on the cytosine etheno derivative (eC). Recently, the increase of its singlet excited state lifetime compared to that of cytosine was reported,[3] which points toward a **potentially increased reactivity**. A model compound containing two eC covalently linked by a trimethylene bridge was synthesized and irradiated to evaluate its photoreactivity under **direct irradiation** or through a **photosensitization** process. Especially, cyclobutane pyrimidine dimer formation, the most common lesion caused by UV light in DNA, was considered taking into account the possibility of a **[2+2] photocycloaddition** involving the double bond at 5,6 of the cytosine moiety or that of the etheno ring.

Formation of edC already means a damage by itself, but its harmfulness could be increased if it can form a CPD-type photoproduct. Thus, the aim of this study is to evaluate the photoreactivity of these etheno adducts, considering especially the formation of new CPD-like lesions.

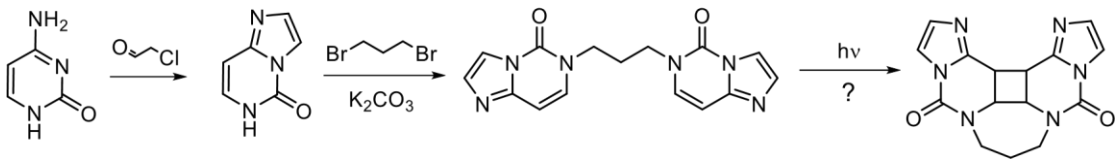

Figure 1: Synthesis and potential photoreactivity of the eC model

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[2] A. Winczura; D. Zdżalik; B. Tudek. *Free Radical Res.* **2012**, 46(4), 442–459

[3] P. Lizondo-Aranda , L. Martínez-Fernández , M.A. Miranda , R. Improta, T. Gustavsson, V. Lhiaubet-Vallet. *J. Phys. Chem. Lett.* **2022,** 13, 1, 251–257

Excited state processes in non-canonical DNA structures: insights from quantum mechanical calculations.

Lara Martinez-Fernandez[1]

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The combination of DNA and UV light has potential beneficial applications for humans as in phototherapies or optoelectronics. Indeed, since in the 80's Ned Seeman (1) envisioned the idea of building artificial structures using DNA, the field of DNA-nanotechnology has grown exponentially. (2) DNA-based devices usually rely on fluorescence and /or energy transfer mechanisms. However, canonical nucleobases, the building blocks of DNA, are almost nonfluorescent and not involved in energy transfer processes. Then, the design of an ideal non canonical DNA structure with optimal optical properties has become in the recent years a hot topic.

With this aim in mind, we have characterized the main excited state processes driven by light absorption in non canonical DNA structures (I-motifs, guanine quadruplex (G4) or metallo-DNA, see Figure 1) using quantum chemical calculations. In detail, some of these structures present stable and characteristic charge transfer excited states (3) that could be the precursors of charge transport mechanisms, whereas in other cases very reactive triplet states are populated. (4)

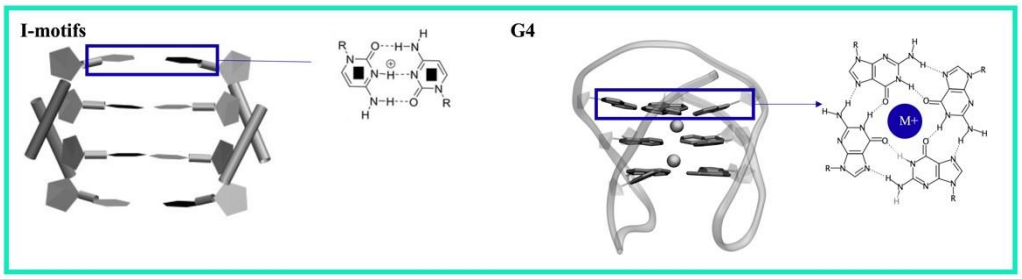

Figure 1. Examples for I-motifs and G-quadruplex structures.

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Parallel symposia

Thursday August 31

Morning

Photodamage and phototoxicity: a mechanistic view

Invited speakers:

IL92 Inmaculada Andreu (València, Spain)

Photobehavior of anticancer drugs: from solution to cells

IL93 Salvatore Sortino (Catania, Italy)

Red light-photosensitised NO release and its monitoring in cancer cells with biodegradable polymeric nanoparticles

IL94 Giorgia Miolo (Padova, Italy)

Photostability of therapeutic monoclonal antibodies in formulation and in saline or glucose solutions for parenteral administration: is their activity compromised by light?

IL95 Andrés Thomas (La Plata, Argentina)

The chemical nature of the photosensitizer and the lipid composition as key factors in the photoinduced damage to biomembranes

Oral communications:

OC96 Lohanna De Faria Lopes: Photobiological evaluation of FICZ and ICZ

OC97 Sylwia Kacprzac: EPR spectroscopy and photochemical processes: an enlightening experience

OC98 Peter Alshede Philipsen: Action spectrum for cyclobutane pyrimidine dimer formation in ex-vivo human skin irradiated with UV-LEDs and measured by quantitative HPLC-MS/MS

OC99 Márcia Franco: Photobiological evaluation of DO15 in deuterated reinforced membranes

Photobehavior of anticancer drugs: from solution to the cells

Inmaculada Andreu[1,2]

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2 Unidad Mixta de Investigación UPV- IIS La Fe, Hospital Universitari i Politècnic La Fe, Valencia, Spain

Anticancer drugs, also known as chemotherapy agents, treat cancer by targeting and destroying cancer cells or inhibiting their growth. In this context, targeted therapies constitute a specific category designed to specifically target molecules or pathways that play a crucial role in the growth and progression of cancer cells. Two examples of these are tyrosine kinase inhibitors (TKIs)[1] and Poly(ADP-ribose) polymerase inhibitors (PARPIs)[2]. Thus, the mechanism of TKIs involves the inhibition of specific tyrosine kinases, disrupting the signaling pathways that facilitate the growth of cancer cells. Conversely, PARPIs operate by blocking the activity of PARP enzymes, hindering the repair of DNA single-strand breaks. Both TKIs and PARPIs are illustrations of precision medicine in cancer treatment, providing more personalized and potentially more effective therapies than traditional chemotherapy; however, they are not devoid of adverse reactions. Concerning this, cutaneous reactions are common in drugs containing chromophores capable of absorbing in the UVA region, as they are intensified with sunlight exposure. Therefore, these side effects could be attributed, in part, to the damage to cellular biomolecules mediated by radicals or reactive oxygen species generated from excited states.

Here, the photobehavior of some TKIs (such as gefitinib[3]) and PARPIs (such as rucaparib[4] and talazoparib) has been investigated, including photophysical and *in vitro* photobiological studies. Moreover, we have also explored the drug biotransformation process[5], which is generally associated to decrease toxicity. However, in some cases, the metabolites may exhibit higher phototoxicity than the parent drug.

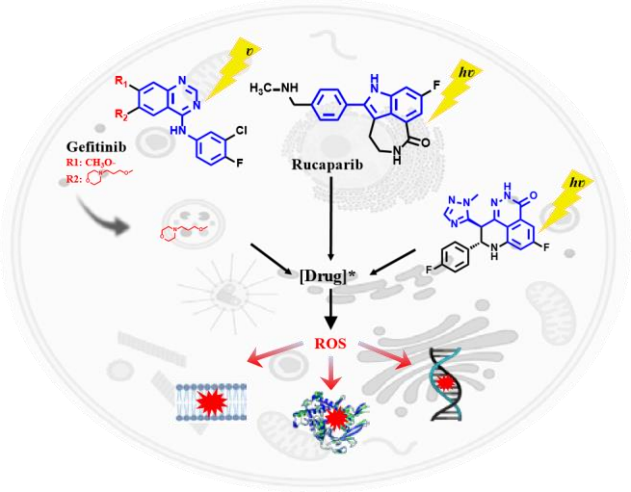

Figure 1. Photosensitization to cellular biomolecules by anticancer drugs

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Acknowledgements. This work was supported by the Spanish government (Grant PID2020-115010RB-I00)

Red light-photosensitized NO release and its monitoring in cancer cells with biodegradable polymeric nanoparticles

Cristina Parisi,[1] Aurore Fraix,[1] Giuseppe Longobardi,[2] Claudia Conte,[2] Arianna Pastore,[2] Mariano Stornaiuolo,[2] Adriana C. E. Graziano,[1] Marta E. Alberto,[3] Antonio Francés-Monerris,[4] Fabiana Quaglia and Salvatore Sortino[1]

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[2] *Department of Pharmacy, University of Napoli Federico II, I-80131, Napoli, Italy*
[3] *Dipartimento di Chimica e Tecnologie Chimiche, Università della Calabria, Arcavacata di Rende, I- 87036, Italy*
[4] *Institut de Ciència Molecular, Universitat de València, 46071 València, Spain*

The multiple role NO plays in a variety of physiological and pathophysiological processes has made the development of precursors and strategies to activate NO release by excitation in the therapeutic window highly desirable and challenging. Herein, we demonstrate that red light excitation of the photosensitizer Verteporfin (VTP) activates NO release from an otherwise blue-light activatable NO photodonor (NBFNO) with an improvement of about 300 nm towards more biocompatible wavelengths. NO photorelease is photosensitized by the lowest triplet state of the PS, more likely by a catalytic photoinduced electron transfer. This process occurs effectively and almost independently by the presence of oxygen when the water insoluble VTP and NBF-NO are co-entrapped within water-dispersible, biodegradable polymeric nanoparticles (NPs) of mPEG-PCL. Moreover, the ideal spectroscopic prerequisites and the restricted environment of the NPs permit the green-fluorescent co-product formed concomitantly to NO photorelease (NBF) to communicate with VTP *via* Förster Resonance Energy Transfer. This leads to an enhancement of the typical red emission of the PS offering the *mechanism* possibility of a double col-our optical reporter useful for the real-time monitoring of the NO release through fluorescence techniques. The suitability of this strategy applied to the polymeric NPs as potential nanotherapeutics was evaluated through biological tests performed by using HepG2 hepatocarcinoma and A375 melanoma cancer cell lines. Fluorescence investigation in cells and cell viabil-ity experiments demonstrate the occurrence of the NO release under one-photon red light illumination also in the biological environment. This confirms that the adopted strategy provides a valuable tool for generating NO from an already available NOPD, otherwise activatable with the poorly biocompatible blue light, without requiring any chemical modification and the use of sophisticated irradiation sources

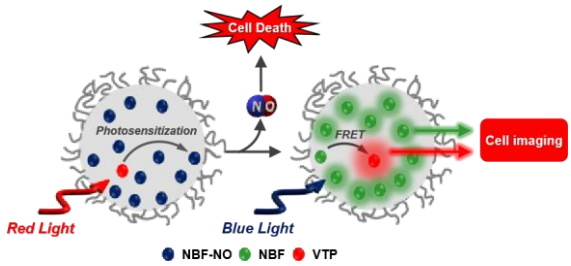

***Figure 1**. Photosensitized NO release and its monitoring*

Photostability of therapeutic monoclonal antibodies in formulation and in saline or glucose solutions for parenteral administration: is their activity compromised by light?

Elisabetta De Diana[1], Marina Coppola[2], Luca Menilli[1], Patrizia Polverino de Laureto[1], Giorgia Miolo[1]

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[2]Veneto Institute of Oncology – IRCCS, Via Gattamelata 64, Padova, Italy

Monoclonal antibodies (mAbs) have rapidly escalated as biopharmaceuticals into cancer treatments in these last years, mainly for their target specificity and stimulation of reliable anti-tumoral responses [1]. During their real-life, they are potentially unstable macromolecules under shaking, temperature fluctuations, humidity, and indoor and outdoor light exposure. All these stressors can occur throughout all stages of mAbs production, transport, storage, handling, and administration. It is important to highlight that the physical and chemical modification of mAbs can lead not only to the loss of their bioactivity, but also to the enhancement of their immunogenicity with increasing risks of severe hypersensitivity reactions [2]. Additionally, mAbs administered intravenously are diluted in 0.9% NaCl or in 5% glucose solutions and consequently the excipients are diluted too decreasing their specific role of protection, i.e., against light modifications. The physicochemical properties and the rate of formation of non-native aggregates are therefore possibly influenced [3]. The photostability of various therapeutic mAbs has been studied. The physicochemical modifications and the biological activity after the light stressor are here reported, with particular attention on the diluting solutions used for its administration to patients.

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This work has received support from the EU/EFPIA Innovative Medicines Initiative Joint Undertaking (H2020-JTI-IMI2) RealHOPE grant n°101007939.

The chemical nature of the photosensitizer and the lipid composition as key factors in the photoinduced damage to biomembranes

Mariana Vignoni[1], José Luis Fonseca[1], María José Sosa[1], María Noel Urrutia[1], Gonzalo Cosa[2], Rosangela Itri[4], Mauricio S. Baptista[3], Andrés H. Thomas[1]

1 INIFTA, Universidad Nacional de La Plata, CONICET, La Plata, Argentina.

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4 Departamento de Física Aplicada, Instituto de Física, Universidade de São Paulo, São Paulo, Brasil.

All unsaturated lipids are targets of oxidative damage, which can occur by photosensitized oxidation. In the case of vesicles dispersions, if the photosensitizer is lipophilic, an association with a biomembrane is expected and the oxidation might be much faster than that caused by hydrophilic photosensitizers that remain in the aqueous phase. Pterins, lumazines and flavins are natural hydrophilic compounds that efficiently photosensitize the oxidation of DNA, proteins and other biomolecules in aqueous solutions. These photosensitizers do not bind to phospholipid membranes. In the search of new compounds that retain the photosensitizing properties of the hydrophilic precursors and, at the same time, are able to bind to biomembranes, a set of decyl-derivatives were synthesized and studied. Pterin (Ptr), lumazine (Lum) and riboflavin (Rf) were chosen as model compounds. Conjugation of a decyl chain to the photosensitizer moiety enables its facile intercalation in phospholipid vesicles. Upon UVA irradiation of vesicles in the presence of a photosensitizer, lipid oxidation takes place, leading to the formation of many oxidized products. The rate of photooxidation depends on the lipophilicity of the photosensitizer, but the relationship is not straightforward. The initial oxidation photoproducts undergo conversion into short-chain secondary products most likely due to further photosensitized processes. These short-chain oxidized lipids are responsible for destabilizing the phospholipid bilayer and promoting membrane leakage. The efficiency of photoinduced permeabilization depends on the composition of the membrane more than on the photosensitizer.

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A. Vignoni *et al. Dyes Pigm.*, **187**, 109131 (2021).

Photobiological evaluation of FICZ and ICZ

Lopes, L. F.[1]; Martins, L. M. O. S.[2]; Palomino, C. M. V.[1]; Wondrak, G. [2]; Baptista, M. S. [1]

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[2]*Departament of Pharmacology and Toxicology, RK Coit College of Pharmacy, The University of Arizona, USA*

INTRODUCTION: Endogenous photosensitizers (EP) are responsible to absorb solar photons and to cause oxidative stress in the excess of sun exposure [1,2]. Among the EP present in the skin, the tryptophanderived photoproduct 6-formylindole [3,2-b] carbazole (FICZ) (Figure 1A) has recently been shown to be a potent EP, besides being an agonist of the aryl hydrocarbon receptor (AhR) [2,[3]]. To understand the possible synergistic roles played by the activation of AhR and by photosensitized oxidation, we examined the consequences of irradiating keratinocytes (HaCaT cells) previously incubated with FICZ and with the indole [3,2-b] carbazole (ICZ), which is not a typical EP and does not efficiently form triplet species and singlet oxygen (figure 1B). **OBJECTIVES:** Understanding the relationship between the AhR and the photosensization. **METHODOLOGY**: The quantum yield of absolute fluorescence emission (Φf) was obtained with excitation source at 390nm and 410nm for FICZ and ICZ, respectively. The quantum yield of singlet oxygen (ΦΔ) was determined using a NIR fluorometer (SHB) with a 400 nm LED as the excitation source. FLIM was performed using a PicoQuant time 200. Biological assays included cell viability of the FICZ/UVA co-treatment by MTT assay and immunofluorescence to visualize AhR activation. **RESULTS:** For FICZ, the ΦΔ was 0.53 and the absolute Φf was 26.3%, while for ICZ the values of ΦΔ and Φf were 0.05 and 24.4%, respectively. The fluorescence lifetime is 4.97ns and 7.74ns for FICZ and ICZ, respectively. Biological effects on the tested cell line (HaCaT) include phototoxicity at nanomolar concentration in addition to activation and sustaining of AhR translocation upon FICZ/UVA co-treatment (figure 1C).

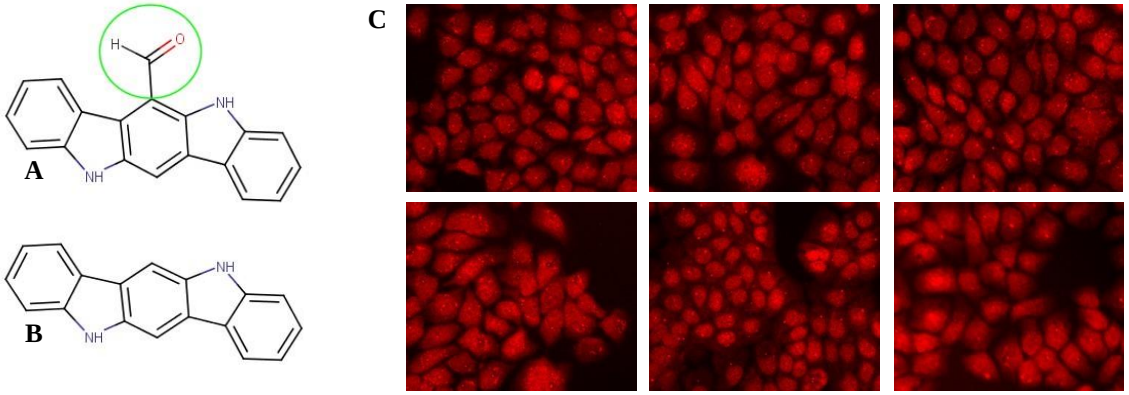

Figure 1. Molecular structure of FICZ (A) and ICZ (B). Activation and translocation of AhR after co-treatment FICZ/UVA or ICZ/UVA in Hacat cells. PSs incubation time was 4 hours and irradiation time 30min. Cells were fixed, permeabilized and stained with Recombinant Alexa Fluor® 647 Anti-Aryl hydrocarbon Receptor antibody 1 hour after PS/UVA co-treatment. The white arrows indicate high marking delimitation in the cell nucleus.

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EPR spectroscopy and photochemical processes – an enlightening experience

Thilo Hetzke, Manuela Liberi, Sylwia Kacprzak

Bruker Biospin

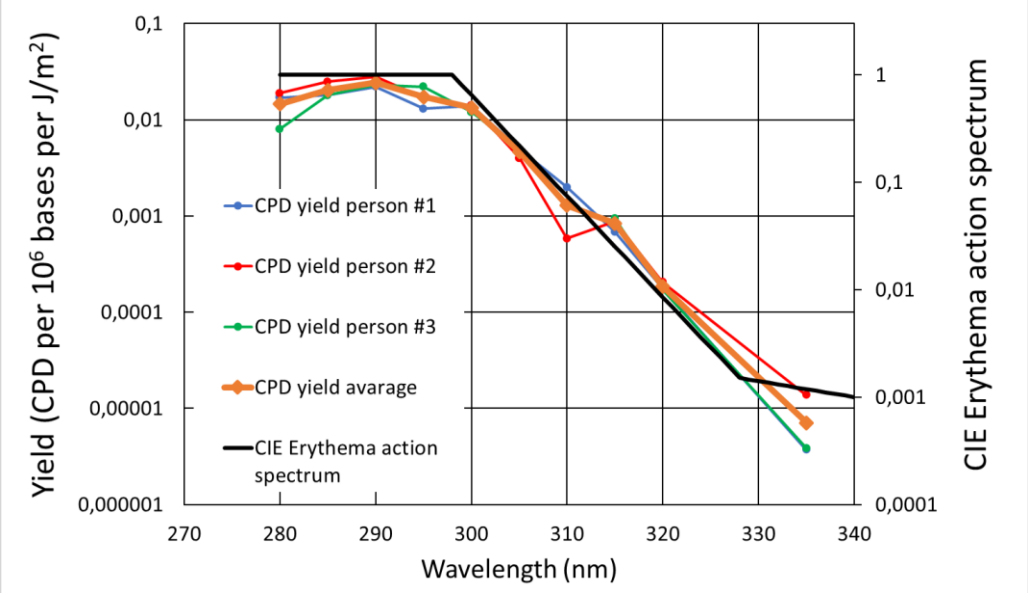
Electron paramagnetic resonance spectroscopy (EPR or ESR) is a non-invasive technique for direct detection of unpaired electron spins. Species that are typically observed with EPR spectroscopy are organic radicals, photoexcited triplet radical states, reactive oxygen species (ROS), and transition metals. In this oral presentation, a brief introduction to the basics of EPR spectroscopy will be given, followed by examples showing how EPR and its unique properties can contribute to understanding photochemical reactions. Applications covered in this presentation will include effects of transition metals on photosystem 2, how the phytotoxic air pollutant ozone affects the photosynthetic apparatus of plants, and spin-trapping of ROS generated by phototoxicity-inducing agents.

Action spectrum for cyclobutane pyrimidine dimer formation in ex-vivo human skin irradiated with UV-LEDs and measured by quantitative HPLC-MS/MS

PA Philipsen[1], TN Gillings[1], T Douki[2], Y Ou[3], PM Petersen[3], J Jakobsen[4], CM Lerche[1,5]

1 Department of Dermatology, Copenhagen University Hospital – Bispebjerg, Denmark |2 Univ. Grenoble Alpes, CEA, CNRS, IRIG, SyMMES, France | 3 Department of Photonics Engineering, Technical University of Denmark, Denmark. |4 National Food Institute, Technical University of Denmark, Denmark. |5 Department of Pharmacy, University of Copenhagen, Denmark.

Ultraviolet (UV) radiation of human skin causes DNA damage. The most common are cyclobutane pyrimidine dimers (CPDs). The amount of CPDs after UV is dependent on wavelength and we aimed to accurately quantify the CPD formation. We have obtained excess waistband skin after it was surgically removed from 3 persons. From each person's skin tissue 41 biopsies were prepared: One non-irradiated control and 40 irradiated with one of 10 UV-LEDs with wavelengths: 280, 285, 290, 295, 300, 305, 310, 315, 320 or 335 nm. For each wavelength, 4 doses with linear increments were given, with a total of 40 irradiated biopsies per skin tissue. Quantification of CPDs in the skin (n=123 in total) was done by HPLCMS/MS (1). For each wavelength, a linear dose response was calculated, and the regression slopes are presented in the figure as a CPD action spectrum. The action spectra have peak at 290 nm with a yield of 24 CPDs per 10^9 bases per J/m^2 and have a close resemblance to the CIE erythema action spectra (2,3).



Human CPD action spectrum based on skin tissue from 3 persons compared to CIE erythema action spectrum.

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Photobiological evaluation of DO15 in deuterated reinforced membranes

Franco Freire, M. S.[1]; Donatto, M.;[2] López M.A.H.[1] Caldeira C.[1] Itri, R[2]; Shchepinov. M. S.[3]; Baptista, M. S.[1]

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2 *Department of Applied Physics, Institute of Physics, The University of São Paulo, São Paulo, Brazil*
3 *Retrotope Inc., 4300 El Camino Real, Suite 201, Los Altos, CA 94022*

Introduction: PUFAs are the main constituents of membrane bilayers however, because of their multiple conjugated double bonds, they are highly susceptible to lipid peroxidation (LPO). [1,2] Bis-allylic hydrogen abstractions trigger radical chain reactions, leading to rearrangements and scissions and resulting in carbonyl compounds and toxic species associated with diseases such as Parkinson's and Alzheimer's neuro-degenerations, hypertension and cancer.[3-6] The isotopic substitution of bis-allylic hydrogens to deuterium gives rise the D-PUFAs, slowing down the cleavage of the CD bond, as compared to C-H cleavage. **Objective:** To evaluate the lipid membrane protection by deuterium reinforced PUFAs, we investigated the photooxidative damage induced by the photoactivation of DO15 in membrane mimics and HaCaT cells previously incubated with D-PUFA [8]. **Methods**: Biological assays included cell viability (MTT, AquaBluer and Neutral red), mitochondrial function/dysfunction (measured by mitochondrial oxygen consumption rate (OCR) using Seahorse Extracellular Flux system) of HaCaT cells previously incubated with DO15 and membrane leakage assays in Giant Unilamellar Vesicles (GUVs), assessed by optical phase-contrast intensity observations. **Results:** The viability of deuterium reinforced cells were significantly higher than that of control cells (incubated with DO15 and irradiated in DMEM), D-PUFAs supplemented cells shown the same proton leak as control, but they have significantly higher production of ATP. GUVs made of H-Lin-PC were stained with DO15 at 1µM and were followed in the microscopy under irradiation with red light. In the presence of 20% of D_2-Lin-PC the increase in the membrane area (A/A_0), which is proportional to the accumulation of lipid hydroperoxides, was substantially delayed as well as the membrane leakage, which was evaluated by the membrane contrast (Figure 1). **Conclusion:** The introduction of D-PUFA into membrane constitute a promising tool against LPO, presenting a protective mechanism from both type I and II photosensitized oxidation mechanisms.[9]

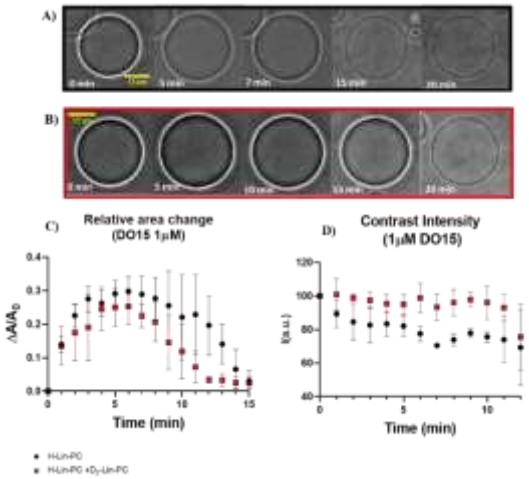

Figure 1- Profiles of contrast decay and area change of H-Lin-PC (**A**, **C** and **D**) and H-Lin-PC + D_2-Lin-PC. (**B**, **C** and **D**).

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Combination therapies or PDT in combinations

Invited speakers:

IL96 Kristian Berg (Oslo, Norway)

Photochemical internalization (PCI): a photodynamic combination therapy for improved drug delivery to solid tumors

IL97 Anne-Laure Bulin (Grenoble, France)

Nanoscintillators for X-ray induced photodynamic therapy: new insights on this complex mechanism Oral communications:

OC100 Luca Menilli: Bioresponsive Pheophorbide A and Paclitaxel prodrug nanoparticles for targeted
OC101 Sofia Joaquinito: New Developments on PhotoChemical Internalization using Porphyrin derivatives

OC102 Mans Broekgaarden: Unraveling the effects of photochemical internalization of oxaliplatin by X-ray fluorescence-based elemental imaging

OC103 Robert Edkin: Stimulated Raman scattering microscopy to induce and image cell death using dual-action photodynamic and photothermal phthalocyanine photosensitizers

OC104 Sarah Stelse-Masson: Using nanoscintillators to improve the efficacy of radiotherapy in pancreatic cancer

OC105 Marine Labro: Photo-generated diazonia for an anticancer therapy using light

cancer treatment

OC106 Pål Kristian Selbo: Promising anti-carcinoma effects of ATRA + PDT

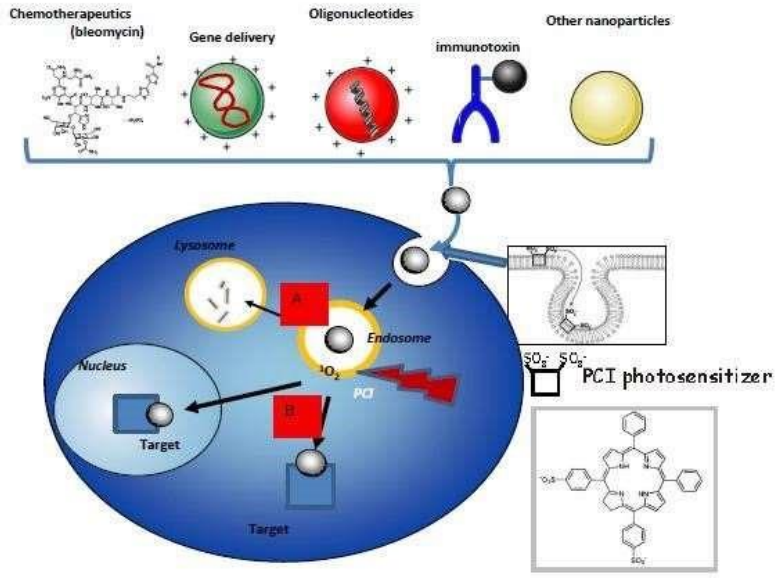
OC107 Bernhard Spingler: BODIPY-Based Photothermal Agents with Unprecedented Phototoxic Indexes under Normoxic and Hypoxic Conditions

Photochemical internalization (PCI) – a photodynamic combination therapy for improved drug delivery to solid tumors

Kristian Berg, Anette Weyergang, and Pål Kristian Selbo

Department of Radiation Biology, Institute for Cancer Research, Comprehensive Cancer Center, Oslo University Hospital - Radium Hospital, Oslo, Norway

Photochemical internalisation (PCI) is a novel technology for release of endocytosed macromolecules into the cytosol. The technology is based on the use of photosensitizers located in endocytic vesicles that upon activation by light induce rupture of the endocytic vesicles and thereby release of the macromolecules into the cytosol. PCI has been shown to enhance the biological activity of a large variety of macromolecules and other molecules that do not readily penetrate the plasma membrane, including type I ribosomeinactivating proteins (RIPs), gene-encoding plasmids, adenovirus, oligonucleotides and some chemotherapeutic agent, such as bleomycin. Novel recombinant protein toxins have been developed for activation by PCI. The PCI treatment has been found to induce vascular shutdown and strong inflammatory effects that may be utilized to stimulate anti-tumor immunity and cancer vaccination. An update on the development of the PCI technology towards preclinical evaluation and clinical implementation will be presented.



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Nanoscintillators for X-ray induced photodynamic therapy: new insights on this complex mechanism

Anne-Laure Bulin[1]

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Photodynamic therapy (PDT) is intrinsically restricted by the shallow penetration of light in tissue and can therefore not be induced in deep tissue without invasive strategies [1]. An elegant non-invasive approach to overcome this limitation consists in conjugating the photosensitizers to radioluminescent nanomaterials also called nanoscintillators, that behave as light sources upon irradiation by the X-rays of radiotherapy.

Upon X-ray irradiation, nanoscintillators are "switched on" and emit light that can subsequently excite the photosensitizer and induce PDT [2]. As X-rays penetrate deeply in tissues, radioluminescence can activate PDT non-invasively at depth and without being restricted by large tumor volumes and optical shielding by blood vessels. The feasibility of exciting photosensitizers using nanoscintillators has been demonstrated by us and others [3–5]. However, there is a mismatch between a low theoretically expected efficacy of radioluminescence-induced PDT and the experimentally measured efficacy that is much higher. Thus, it appears critical to understand the origin of the observed efficacy that may stem from several therapeutic contributions.

In this presentation, I will discuss various radiotherapeutic effects that can be induced by nanoscintillators when used to induce deep-tissue photodynamic therapy upon X-ray irradiation [6]. These effects may strongly contribute to the overall treatment efficacy and therefore need to be considered when designing future nanomaterials for X-ray induced PDT.

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Bioresponsive Pheophorbide A and Paclitaxel prodrug nanoparticles for targeted cancer treatment

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Chemotherapy remains a crucial approach in cancer treatment, but its efficacy is hindered by significant side effects. Challenges, including the inadequate delivery of chemotherapeutic drugs to the tumor site and the emergence of drug resistance, highlight the need for innovative therapeutic strategies. Combination therapy has proven to be highly promising in fighting cancer, and the recent advances in nanotechnology have enabled the development of nanosystems capable of precisely regulate the ratio of multiple therapeutic agents together with their selective transport to target sites. However, despite the use of delivery systems, the premature release of drugs still leads to the occurrence of adverse effects. In recent years, the development of chemotherapeutic prodrugs allowed a better spatial control of their activity, in response to specific stimuli (ROS, high GSH concentration, pH, etc.).

In this study, the in vitro antitumoral efficacy of tumor microenvironment (TME) responsive nanoparticles exclusively composed of a paclitaxel prodrug (PTX_2S) and the photosensitizer pheophorbide A (PheoA) is reported on two different cancer cell lines (MDA-MB-231 and SK-OV-3) for chemo-PDT application. The increased toxicity observed in MDA-MB-231 cells exposed to a simulated TME (10 mM glutathione, GSH) and then treated with PTX_2S indicated that a more reductive environment promotes a greater extent of PTX release and consequently cell death. The same scenario was observed when cells exposed to reductive microenvironment were irradiated with red light, thus indicating that ROS further promotes prodrug disassembly. The combination of PTX_2S and PheoA into a single nanosystem allowed a 30-fold PTX dose reduction and a 3-fold dose reduction of PheoA.

Subsequently, a second-generation of nanoparticles was developed, incorporating a paclitaxel prodrug that binds to human serum albumin (HSA), e.g., MAL-PTX2S, and PheoA. These nanoparticles demonstrated increased uptake in MDA-MB-231 and MCF7 breast cancer cell lines, as well as a more potent inhibition of cell viability in both cell monolayers and 3D spheroids upon irradiation. Overall, these findings highlight the ability of HSA-binding nanocarriers to selectively internalize drugs in cancer cells, thus presenting a promising approach for targeted drug delivery.

New Developments on PhotoChemical Internalization using Porphyrin derivatives

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Cancer is one of the leading causes of death worldwide, with breast cancer as the second most common cancer in the world [1].

Photochemical internalization (PCI) is as a novel PDT-based technology for cytosolic delivery of endocytosed macromolecules. The irradiation of light allows for the rupture of the membrane of endocytic vesicles and release of the drug to the cytosol [2]. This allows for the use of a lower dose of PS which acts synergistically with the drug to produce an effective tumor cell toxicity. Macromolecules, such as type I ribosome-inactivating proteins (RIP), are being tested as anti-cancer agents, however one of the disadvantages is their lack of capacity to penetrate cellular membranes [3].

The molecular characteristics of the PS are of utmost importance for the success of PCI. Both porphyrins, chlorins and phthalocyanines have been tested for PCI, but none have been approved for clinical use [5,6].

For this purpose, a novel PS was synthesized, characterized and tested for PDT and PCItreatment of breast cancer cell line using recombinant gelonin as the type I RIP anti-cancer drug. The prepared compounds showed great efficiency in acting as PS in both PDT and PCI treatments. In this communication, the main results of PDT and PCI in breast tumor cells will be presented and discussed.

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Unraveling the effects of photochemical internalization of oxaliplatin by X-ray fluorescence-based elemental imaging

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Oxaliplatin is widely used in treatment of pancreatic cancer (PDAC), but its high toxicity gives value to new strategies that enhance its efficacy and safety [1,2]. As oxaliplatin is highly hydrophilic, its efficacy may be enhanced by Photochemical internalization (PCI), which can facilitate the endolysosomal release and increased efficacy of various hydrophilic/ macromolecular chemotherapeutics [3]. Indeed, photodynamic therapy enhances oxaliplatin efficacy [4], yet whether this occurs via PCI has never been investigated. This work aims to investigate the effects of low-dose PDT on the intracellular oxaliplatin distribution patterns.

In both 2D cell cultures and 3D cultures of MIA PaCa-2 and PANC-1 cell lines, the efficacy of oxaliplatin exhibited >4-fold reduction in presence of endocytosis inhibitors. Low-dose PDT (0.5 J/cm2), using a PEGylated liposome formulation of verteporfin, significantly increased oxaliplatin efficacies in all models. Elemental X-ray fluorescence imaging revealed lysosomal destruction by low-dose PDT (0.5 J/cm^2), with elevated intracellular Pt concentrations (Figure 1), with Fe being a potential therapeutic cofactor.

In conclusion, we present for the first time, that PDT/PCI can induce lysosomal release of oxaliplatin, which holds promise to increase the safety and efficacy of this widely used chemotherapeutic.

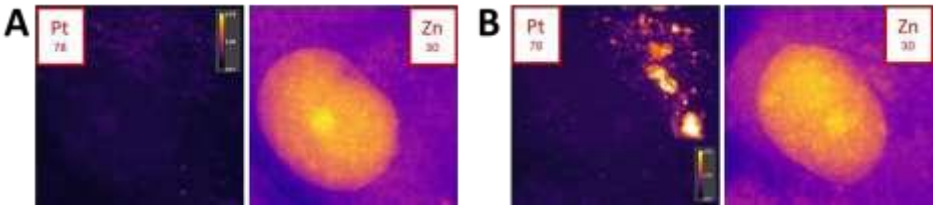

Figure 1. Calibrated heatmaps (ng/mL) obtained by X-ray fluorescence elemental imaging on PANC-1 cells shows the intracellular Pt distribution before (A), and after PCI (B). Zn images are included as a physiological marker for nuclei.

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Stimulated Raman scattering microscopy to induce and image cell death using dual-action photodynamic and photothermal phthalocyanine photosensitizers

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Phthalocyanines (Pcs) are efficient photosensitizers of singlet oxygen and are therefore promising candidates for use in photodynamic therapy (PDT).[1] More recently Pcs have also been shown to be applicable in photothermal therapy (PTT), wherein photoinduced heating occurs following efficient nonradiative decay.[2] PTT is a useful alternative for the treatment of hypoxic (low [O_2]) tumors.

We have synthesized a series of zinc and copper Pcs using a method that overcomes the common trade off in Pc synthesis of regioisomer purity or desired functionalization to give selectively C_{4h}-symmetric Pcs.[3,4] Irradiation of these Pcs in an aggregated state using near infrared light (785 nm) induces a photothermal temperature rise of +25 °C in bulk aqueous solution with photothermal efficiencies >30%, and in the case of the zinc Pcs, additional singlet-oxygen photosensitization by residual monomer. These photosensitizers are therefore potentially useful dual-action phototherapeutics.

To observe their phototherapeutic effect in a model cancer cell line (HeLa), we irradiated the Pcs and imaged cell death simultaneously using a stimulated Raman scattering (SRS) microscope. We observe photoactivated cell blebbing, a common indicator of cell death by apoptosis, and morphological changes in real time, even at 0.5 µM Pc.

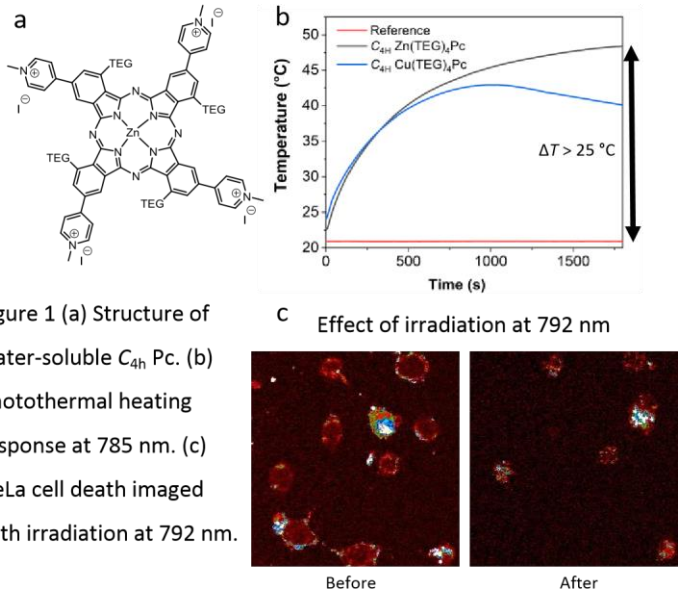

Figure 1 (a) Structure of water-soluble C_{4h} Pc. (b) Photothermal heating response at 785 nm. (c) HeLa cell death imaged with irradiation at 792 nm.

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Using nanoscintillators to improve the efficacy of radiotherapy in pancreatic cancer

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Pancreatic cancer is associated with a poor prognosis despite multimodal treatments consisting in surgery, chemotherapy and radiotherapy. Photodynamic therapy (PDT) is a promising treatment that aims to activate photosensitizers with light in order to induce local cell damage. However, its use for treating deep tumors, including pancreatic tumors, is limited because of the low penetration depth of light in tissues. To overcome this limitation, it has been proposed to use nanoscintillators (NS) that down-convert ionizing radiation such as X-rays used in radiotherapy, into UV-visible photons. These nanoparticles could improve the efficacy of radiotherapy through at least three effects: the radiation dose-enhancement effect[1] that is due to the accumulation of heavy elements in the vicinity of tumors, the emission of UV-C photons that increase direct DNA damage, and the activation of photodynamic therapy by X-rays (X-PDT)[2]. The aim of our project is to conjugate NS with appropriate photosensitizers to achieve PDT during radiotherapy.

When developing nanoscintillators for biological applications, the choice of the coating is of prime importance as it can modify both the NS toxicity and uptake in cells and microtumors, and thus impact their therapeutic efficacy. Here, we present our results obtained with lanthanum fluoride nanoscintillators doped with cerium (LaF_3:Ce), coated with either polyethylene glycol (PEG) or tripolyphosphate (TPP). We studied the properties of NS on both 2D and 3D culture models of pancreatic tumors, grown in suspension or as adherent microtumors. LaF_3:Ce nanoparticles could not be observed with a conventional confocal microscope, as they can only be excited by X-rays or UV photons. Therefore, we used X-ray fluorescence microscopy (ID16, ESRF, European synchrotron) and transmission electron microscopy (IBS, Institut de Biologie Structurale) to analyze their distribution within the cells, and X-ray fluorescence microtomography (P06, DESY, German synchrotron) to study their accumulation in tumor spheroids.

We observed that NS coating induced significant differences in their aggregation and accumulation in cells and tumor spheroids. We also obtained promising preliminary results regarding their therapeutic efficacy, and are currently investigating the role of the coating on pharmacokinetics and biodistribution using *in vivo* models of pancreatic tumors. Finally, their conjugation with photosensitizers will be investigated to evaluate X-PDT efficacy.

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Photo-generated diazonia for an anticancer therapy using light

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Recently, a photo-triggered intramolecular double nucleophilic aromatic substitution (S_NAr) has been discovered allowing the straightforward synthesis of the new compound **2** suitable for the development of a new anti-cancer treatment. Indeed, diazonia **2** selectively stabilizes G-quadruplexes (G4) structures and generates singlet oxygen (Fig 2.A).[1] In addition, thanks to the fluorescence of the photocyclized product **2**, its generation and localization may be followed up *in cellulo* (Fig 1.B).

Therefore, we are developing an innovative therapeutic strategy inspired by both photodynamic therapy and photopharmacology.[2] After injection of bis-quinoleine **1** to the patient (Fig 1.A), the inactive drug **1** will spread in the whole body and will accumulate specifically in tumor tissues thanks to tumor targeting groups decorating **1** (Fig 2.B). Then, a local irradiation of the tumoral cells will generate the photoreaction (Fig 1). Consequently, a combined therapeutic effect is triggered with the local generation of 1) diazonia **2** that causes apoptose by G-quadruplexes stabilization, 2) two photo-released counter ions chosen as additional chemotherapeutic drugs and 3) singlet oxygen (Fig 1.B).

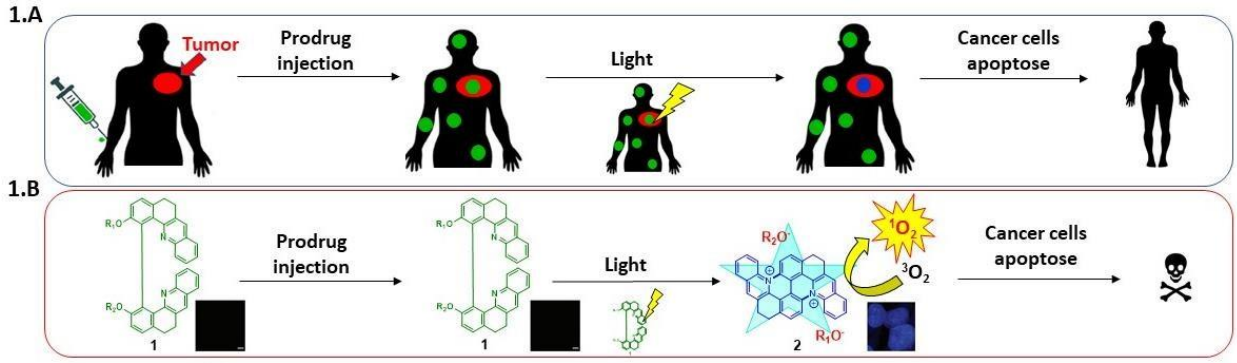

Figure 1: *Pro-drug **1** mode of action as an anticancer therapeutic agent.*

Consequently, we are improving our first-generation pro-drug to increase its biocompatibility and efficiency regarding the photoreaction. Thus, we have developed a family of derivatives of **1** bearing motifs that will improve water-solubility and shift the absorption spectra to the biological window. In addition, we have identified the best photo-labile arms and added tumor-targeting groups or chemotherapeutic agents to combine the therapeutic effects.

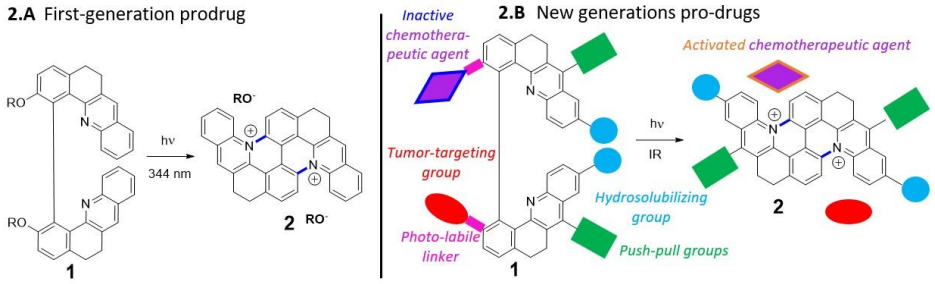

Figure 2: *Design optimisation of pro-drug **1**.*

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Promising anti-carcinoma effects of ATRA + PDT

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The vitamin A metabolite all-trans retinoic acid (ATRA; tretinoin) has anticancer potential. However, lack of clinical success has prevented its approval for solid tumours. Herein, we propose combining short-term low-dose ATRA with fimaporfin-based photodynamic therapy (ATRA+PDT) for the improved treatment of solid cancers. Compared to monotherapies, ATRA+PDT induced synergistic cytotoxic responses including promotion of apoptosis in colon and breast carcinoma cell lines. Neither enhanced activity of alkaline phosphatase (ALP) nor increased expression of CD133 was detected after ATRA treatment indicating that the improved therapeutic effect of ATRA+PDT is independent of the differentiation state of the cancer cells. In the human colorectal adenocarcinoma cell line HT-29, the effect of ATRA+PDT on gene expression was evaluated by RNA sequencing (RNAseq). We identified 1129 differentially expressed genes (DEGs) after ATRA+PDT compared to PDT. Ingenuity Pathway Analysis (IPA) predicted the unfolded protein response (UPR), interferon (IFN) signaling and retinoic acid-mediated apoptosis signaling as strongly activated canonical pathways after ATRA+PDT compared to PDT. A validation of the RNA-sec data by RT-qPCR revealed that ATRA+PDT elevated mRNA expression of early growth response 1 (EGR1) and strongly the stressinduced activating transcription factor 3 (ATF3), of which was confirmed on the protein level. In addition, ATRA+PDT abolished mRNA expression of regenerating islet-derived protein 4 (REG4). During the first 20 days post-ATRA+PDT, we obtained significant anti-tumour responses in HT-29 xenografts, including complete responses in 2/5 mice. In conclusion, ATRA+PDT represent a novel combination therapy for solid tumours that should be further tested in immunocompetent preclinical models.

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BODIPY-Based Photothermal Agents with Unprecedented Phototoxic Indexes under Normoxic and Hypoxic Conditions

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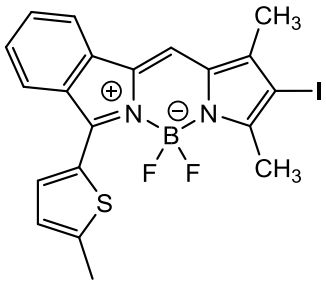
We will report about novel, easily accessible BODIPY photosensitizer (see Figure) for cancer treatment.[1] In contrast to established photodynamic therapy (PDT) agents [2, 3], these BODIPY-based compounds show photothermal activity and their cytotoxicity is independent of reactive oxygen species (ROS). The agents show high toxicity upon light irradiation and low dark toxicity in different cancer cell lines in 2D culture as well as in 3D multicellular tumour spheroids (MCTSs). The ratio of dark to light toxicity (phototoxic index, PI [4-9]) of these agents exceeds 830'000 after irradiation with energetically low doses of light at 630 nm. Under hypoxic conditions (0.2% O_2), which are known to limit the efficiency of conventional photosensitizers (PSs) in solid tumours [10], an excellent phototoxic index of 360'000 was observed, indicating a photothermal mechanism of action (MOA). Both phototoxic values are the highest reported to date (compare with [11]).



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Perspectives and controversies in sun care

Invited speakers:

IL98 Maurício da Silva Baptista (São Paulo, Brazil)

Visible light and the skin redoxome

IL99 Sergio Schalka (Cotia, Brazil)

Visible light induced hyperpigmentation

IL100 Indermeet Kohli (Detroit, USA)

Blue light, electronic devices and the skin

IL101 Tasneem Mohammad (Detroit, USA)

National Academies report on environmental and health impact of UV filters

Oral communications:

OC108 Katie M. Dixon: Determining the efficacy of a natural home-made sunscreen promoted by wellness bloggers on social media

OC109 Gwendal Josse: Nuclear and urinary measurements show the efficacy of a SPF50+ sunscreen against DNA photoproducts upon “real-life” recreational exposure

OC110 Arjan van Dijk: Safety valves on the photoproduction of vitamin D: new insights

OC111 Ellen Bruzell: A systematic approach to assess sunscreen use and UV filters in the Norwegian population

Visible light and the skin redoxome

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Redoxome is the network of redox reactions and redox active species that affect the homeostasis of cells and tissues. Due to the intense and constant interaction with external agents, the human skin evolved to host a robust redox signaling framework. The lack of redox regulation causes the accumulation of oxidation end-products being correlated with several skin disorders, including photoaging and skin cancer.(Schalka, Silva et al. 2021) Protection of human skin against sun exposition is a complex issue that involves ambivalent aspects of the interaction of light with tissues. One misconception that has persisted in our society is that visible light is safe to the skin, even though recent data indicate that at least part of the visible spectrum decreases the epidermal barrier function, induces pigmentation in individuals with type IV and V skins and induces inflammatory response.(de Assis, Tonolli et al. 2021) Endogenous molecules absorb UVA and visible light (VL) inducing several photosensitized oxidation reactions, which end-up deregulating the redox homeostasis and causing oxidative distress in skin cells and tissues, inducing the accumulation of glycation and lipid peroxidation end products, which are usually more effective photosensitizers than their respective their precursor molecules.(Chiarelli-Neto, Pavani et al. 2011, Chiarelli-Neto, Ferreira et al. 2014, Tonolli, Chiarelli-Neto et al. 2017, Tonolli, Martins et al. 2020, Tonolli, Baptista et al. 2021) In this lecture, I will analyze the main molecular networks of redox regulation present on the human skin, explain the mechanisms by which endogenous molecules (absorbing either UVA radiation or visible light) cause a dysregulation of the skin redoxome and analyze the consequences to human skin, aiming to propose more comprehensive mechanisms of sun care. The recently released action spectra of VL on keratinocytes (Tonolli, Palomino et al. 2023), showing that the level of phototoxicity follows the order: violet>blue >green>>red light, will be presented and discussed.

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Visible light induced hyperpigmentation

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São Paulo University – SP – Brazil

Head of the Brazilian Consensus on Photoprotection

Chariman of Latin American Consensus on Photoprotection

The presentation will review the effects of visible light on the skin, the relation between visible light and hyperpigmentation and the evidence of the sunscreen use in the prevention of visible light hyperpigmentation.

Blue light, electronic devices, and the skin

Indermeet Kohli, PhD

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The biologic effects of solar visible light, particularly blue light (BL), on the skin have been established. However, it is unclear if this can be generalized to the impact of BL from electronic screen devices. This presentation will discuss available literature regarding clinical effects of BL emitted from electronic devices on human skin. Objective evaluation of the data using the framework established by the Office of Health Assessment and Translation (OHAT) will also be presented.

Perspectives and controversies in suncare: National Academies report on environmental and health impact of UV filters

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Sunscreen is used worldwide for the prevention of cutaneous malignancy, photoaging, and the management of photodermatoses and pigmentary disorders. However, concerns have been raised regarding the potentially deleterious effects of sunscreen filters on human health and the environment. The purpose of this talk is to comprehensively discuss the National Academies of Science, Engineering, and Medicine's review on the effects of sunscreen on aquatic environments as well as the potential implications for human health.

Determining the efficacy of a natural home-made sunscreen promoted by wellness bloggers on social media.

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Topical sunscreens remain an important tool for protection against the damaging effects of ultraviolet radiation (UV). Concomitantly, consumers are increasingly exposed to online information suggesting sunscreen ingredients such as oxybenzone have harmful side effects as well as detrimental effects on coral reefs. This has generated interest in alternative natural homemade sunscreens, most of which lack scientific evidence supporting their efficacy. Natural sunscreen recipes have been published widely by wellness bloggers on social media platforms and are growing in popularity. In this study, we investigated the efficacy of a natural home-made sunscreen (NHSS) promoted by a wellness blogger with over one million followers across multiple social media platforms, comparing it to a commercially available SPF50+ sunscreen. 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 explants were obtained with consent from both male and female patients undergoing elective surgery. Base lotion, NHSS or commercially available SPF50+ sunscreen (2 mg/cm^2) was applied to skin explants 20 minutes prior to solar simulated UV irradiation (20 J/cm^2). NHSS was prepared either three weeks (NHSS3W) or one day (NHSS1D) prior to UV exposure and stored at room temperature in an opaque container. Skin samples were fixed and processed histologically to determine levels of UV-induced DNA damage in the form of cyclobutane pyrimidine dimers (CPDs) and 8-oxo-2'-deoxyguanosine (8oxodG) at 3 h post-UV by immunohistochemistry and image analysis, as well as sunburn cells (SBCs; apoptotic keratinocytes) and epidermal thickness at 24 h post UV. As anticipated, SPF50+ sunscreen significantly ($p<0.0001$) reduced UV-induced DNA damage in the form of CPDs. Both NHSS3W and NHSS1D formulations also significantly ($p<0.001$) reduced CPDs though to a slightly lesser extent than SPF50+. Interestingly, all three sunscreen formulations provided similar levels of protection against 8oxodG. Indeed, many of the NHSS ingredients have known antioxidant effects. Cells harbouring extensive UV-induced DNA damage may undergo apoptosis and are visualized as SBCs with haematoxylin and eosin staining. Only SPF50+ significantly reduced SBCs, while both NHSS formulations did not protect against SBCs. Both NHSS and SPF50+ sunscreens significantly ($p<0.0001$) reduced epidermal thickness compared with base lotion following UV. With increased consumer reliance on readily available health information on social media and online platforms, the potential for conveying nonfactual advice affecting sun protection behaviours is a real concern. While the NHSS provided protection against UV-induced DNA damage under our controlled conditions, further studies are required to determine shelf life and water resistance, and to determine accuracy of formulation with different household measurement utensils and by different consumers. 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 are perceived to cause harmful side-effects and bleaching of coral reefs. It is therefore important to determine the efficacy of such sunscreens to ensure consumers are making informed decisions regarding their sun protection behaviours to avoid skin carcinogenesis.

Nuclear and urinary measurements show the efficacy of a SPF50+ sunscreen against DNA photoproducts upon "real-life" recreational exposure

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2 Univ. Grenoble Alpes, CEA, CNRS, IRIG, SyMMES, F 38000 Grenoble, France

3 Service Recherche Pharmaco-Clinique, Département Recherche Appliquée, Centre R&D Pierre Fabre, Toulouse, France

Sunscreens have been shown to protect against ultraviolet radiation-induced DNA damage in human skin under laboratory conditions. We presently extended these observations to "real-life" conditions in volunteers following their ordinary exposure habits during summer holidays. Volunteers were randomly assigned to a "control group" and an "educated group" supplied with a SPF50+ sunscreen and receiving instructions of use. A questionnaire was used to determine the extent of exposure. No difference in average solar UVR exposure was found between the two groups. DNA photoprotection was first assessed by a novel non-invasive assay based on the quantification of pyrimidine dimers released by DNA repair in urine. Damage were also quantified in the nuclear DNA extracted from the roof of suction blisters collected after recreational exposure. The urinary concentration of photoproducts was significantly higher in the control compared to the educated group. The same trend was observed for the level of photoproducts in the DNA from suction blisters. The unambiguous observation of an efficient photoprotection against DNA damage afforded by sunscreen under "real-life" conditions provides strong support for the efficiency of the sunscreens. In addition, the results validate the use urinary DNA photoproducts as a non-invasive assay applicable to photoprotection.

Safety valves on the photoproduction of vitamin D: new insights

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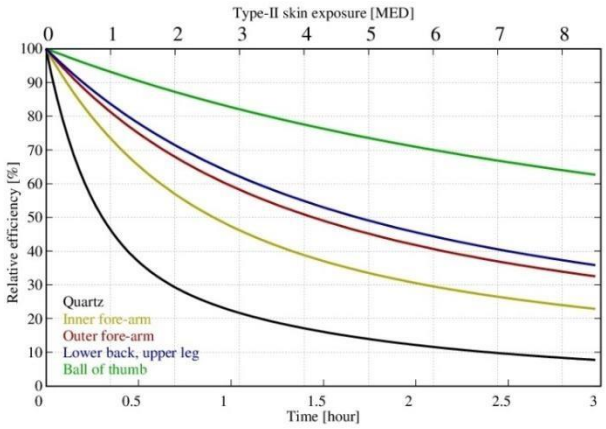
It is common knowledge that the photoproduction of vitamin D, that is associated with prolongued exposure to UV-radiation, does not lead to intoxication (Webb et al, 2021). Experimental evidence has shown that side-reactions in the complex reaction scheme limit the production of the chemical previtamin D. This is often rolled into public health advice to limit sun exposure to less than 1 Minimum Erythema Dose, both to prevent sunburn, and on the premise that further exposure will not benefit vitamin D production. In this presentation, we trace back the evidence behind this statement, which is a set of measurements taken in a quartz vessel. We will show in a model analysis that while photoproduction of vitamin D in human skin is most efficient at sub-erythemal exposures, it is only appreciably attenuated after more extreme, non-physiological exposures, and therefore self-limitation of the photoproduction plays little role in modern everday practice. Evidence for other mechanisms that protect people against hyper-vitaminosis D is given.



Relative efficiency of photoproduction of previtamin D in the course of exposure, when compared with initial photoproduction. Different skin sites (modelled) and a solution of 7DHC in a quartz vessel (measured) are represented.

Reference

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A systematic approach to assess sunscreen use and UV filters in the Norwegian population

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Norway is among the countries with the highest incidence and mortality of skin cancer worldwide. The safety of sunscreen ingredients and use are frequently debated both from human health and environmental perspectives. On this background we performed a systematic assessment of the following: 1) the hazard of and dermal exposure (partly systematic) to the six most frequently used UV filters in sunscreens in Norway: bis-ethyl-hexyloxyphenol methoxyphenyl triazine; butyl methoxydibenzoyl methane; 2-ethylhexyl salicylate; ethylhexyl triazone; octocrylene; and titanium dioxide in nanoform 2) the hazard of sunscreen use, and 3) the protection of sunscreen use against UVinduced adverse effects. Scientific publications and reports up to 2020 were retrieved to assess adverse and protective effects of sunscreen and adverse effects of UV filters. Specific searches were made for data on concentrations and dermal absorption of UV filters and individual user amounts of sunscreens. We developed a method for systematic evaluation of the quality of studies on analysis of UV filter concentrations. Probabilistic methods were used to estimate exposure to each UV filter. Risk of bias in eligible studies and level of evidence for health outcomes were assessed using validity tools (1). Following the publication of the report (2), a new literature search was made. Little to no hazard was associated with exposure to the UV filters. Their concentrations were below the limits set in the EU cosmetics regulative. Thus, the risks related to the evaluated UV filters were considered negligible. Determining the hazards (and thereby risks) associated with sunscreen use were precluded by insufficient evidence. Based on the evidence found that sunscreens protect against certain skin cancers, we concluded that for the general Norwegian population, sunscreen use is beneficial.

Reference

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Pollution and sun exposure

Invited speakers:

IL102 Giuseppe Valacchi (Raleigh, USA)

Skin damage by combined UV and ozone exposure

IL103 Walid Rachidi (Grenoble, France)

UV irradiation, Photo-pollution, Exposome and the human skin health: what Else?

IL104 Valerie Haydont (Aulnay-sous-Bois, France)

Chronic exposure to pollutants photoactivated by UVA1 generates a specific skin response, clearly distinct that those produced by each stresses applied separately

IL105 Patrick Rochette (Quebec, Canada)

Mechanisms underlying the synergistic toxicity of blue light and indenopyrene to retinal cells

Oral communications:

OC112 Maeva Boulée: Degradation of plastic nanoparticles exposed to UV light, toxicological consequences on human intestinal cell models

OC113 Agne Kalnaityte: Effects of CdSe/ZnS-COOH quantum dots on autofluorescence properties and proliferation of Scenedesmus quadricauda microalgae cells: dependence on growth medium composition

OC114 Eloïse Larnac: Photopollution on dermal fibroblasts: a synergistic toxicity effect

Skin damage by combined UV and ozone exposure

Giuseppe Valacchi

Plants for Human Health Institute Animal Science Dept., NC Research Campus Kannapolis, NC, 28081, United States; Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; Kyung Hee University, Department of Food and Nutrition, South Korea. Electronic address: gvalacc@ncsu.edu.

Air pollution represents one of the main risks for both environment and human health. The rapid urbanization has been leading to a continuous release of harmful manmade substances into the atmosphere which are associated to the exacerbation of several pathologies. The skin is the main barrier of our body against the external environment and it is the main target for the outdoor stressors. Among the pollutants, Ozone (O_3) is one of the most toxic, able to initiate oxidative reactions and activate inflammatory response, leading to the onset of several skin conditions. Moreover, skin is daily subjected to the activity of Ultraviolet Radiation which are well known to induce harmful cutaneous effects including skin aging and sunburn. Even though both UV and $O_3$are able to affect the skin homeostasis, very few studies have investigated their possible additive effect. Therefore, in this study we evaluated the effect of the combined exposure of O_3 and UV in inducing skin damage, by exposing human skin explants to UV alone or in combination with O_3 for 4-days. Markers related to inflammation, redox homeostasis and tissue structure were analyzed. Our results demonstrated that O_3 is able to amplify the UV induced skin oxinflammation markers.

UV irradiation, Photo-pollution, Exposome and the human skin health: what Else?

Walid Rachidi

Univ. Grenoble Alpes, CEA, INSERM, IRIG-BGE UA13, 38000 Grenoble, France

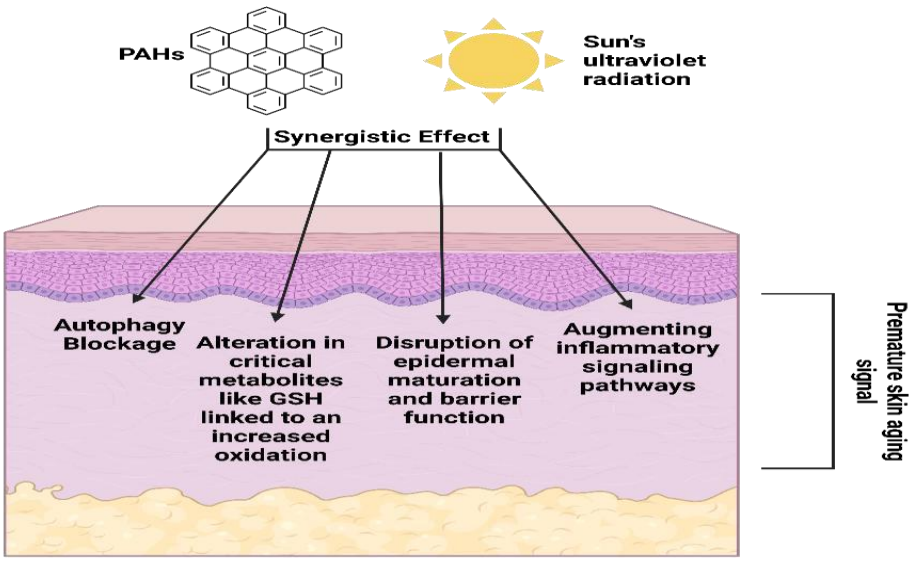
The regeneration of the epidermis is enabled by the activities of interfollicular stem cells and their progenitor cells.

These cells are a direct target of UV radiation which will induce inflammation and DNA damage. The accumulation of DNA damage and the lack of DNA repair (exacerbated by ageing), will result in premature ageing and consequently a decrease in epidermal regeneration. In addition, the skin, as an interface with the external environment, will come into contact with other pollutants which will act in synergy to induce skin anomalies. Therefore, I will talk about the effect of a co-exposure of the skin to UV and a pollutant that is widely answered (Benzo(a)Pyrene). We have very recently shown that this co-exposure significantly decreases the induction of autophagy, a very important cellular process to regenerate cells and oppose the phenomenon of senescence. Knowing the molecular mechanisms responsible for skin ageing due to the exposome is essential for finding preventive or curative strategies against skin ageing.

Reference

Fayyad-Kazan M, Kobaisi F, Nasrallah A, Matarrese P, Fitoussi R, Bourgoin-Voillard S, Seve M, Rachidi W. Effect of Ultraviolet Radiation and Benzo[a]pyrene Co-Exposure on Skin Biology: Autophagy as a Potential Target. Int J Mol Sci. 2023 Mar 20;24(6):5863. doi: 10.3390/ijms24065863. PMID: 36982934; PMCID: PMC10056937.

Figure Synergistic effect of UV abs OAH



Chronic exposure to pollutants photoactivated by UVA1 generates a specific skin response, clearly distinct that those produced by each stresses applied separately.

Valérie Haydont[1], Olivia Zobiri[1], Hélène Zucchi[1], P Perez[1], Ariane Dimitrov[1] and Laurent Marrot[1]

[1] Advanced Research, L'Oréal Research and Innovation, 93600 Aulnay-sous-Bois, France.

Benzo-a-pyrene (BaP) belongs to the polycyclic aromatic hydrocarbon family, and is classified as carcinogenic. BaP is one of the pollutants that diffuse into the atmosphere as fine and ultrafine particles. Its emission sources are mainly incomplete combustion fumes (plant combustion, vehicle exhaust, cigarette smoke, barbecue, etc.). Due to its chemical structure, BaP can be photo-activated by the energy emitted by UVA1 (340-400nm). Its toxicity is then modified. At the interface between the body and the environment, the skin can be affected by pollutants via two types of exposures: 1) topically, at the stratum corneum level, 2) systemic, through the bloodstream. Two models were developed to analyze the consequences of chronic exposure to BaP (photo-activated or not) at the cutaneous level. The first model concerns the quality of epidermal reconstruction. The second model concerns the quality and the organization of the dermal compartment. Experiments were designed to ensure realistic modelling urban exposure. Low BaP concentrations, comparable to those reported for plasmatic contamination, and an UVA1 irradiation corresponding to the UVA1 dose received by the skin in 30 minutes of daylight exposure, were chronically applied.

During epidermal reconstruction, combined exposure to UVA1 and BaP exacerbated the impact of UVA1 exposure alone. A defective maturation of the epidermis was observed, objectified by a perturbed expression of differentiation markers, including filaggrin, and an increase in parakeratosis (1). After 3 weeks of exposure to low doses of BaP, with or without regular UVA1 exposures, papillary fibroblasts showed phenotypic alterations, objectified by an alteration of their ability to organize an extracellular matrix network.

Reference :

(1) Repeated Exposures to UVA1 and Particulate Matter–Associated Pollutants Trigger Epidermal Barrier Dysfunction in Skin Epithelialization Model. Zobiri O, Zucchi H, Dimitrov A, Marrot L. J Invest Dermatol. 2022 Dec;142(12):3331-3335.e8.

Mechanisms underlying the synergistic toxicity of blue light and indenopyrene to retinal cells

Corinne Zinflou[1] and Patrick J Rochette[1,2]

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2 Département d'Ophtalmologie et ORL- chirurgie cervico-faciale, Université Laval, Québec, QC, Canada

Tobacco smoking and high-energy visible blue (HEV; 400-500 nm) light exposure are major environmental risk factors for age-related macular degeneration (AMD), the leading cause of blindness in industrialized countries. Individually, they have been shown to cause damage to the RPE. We have identified indenopyrene (IcdP), an important organic combustion-derived polycyclic aromatic hydrocarbon (PAH), which can accumulate in the retina, as an exogenous HEV light photosensitizer. HEVlight absorption by nanomolar concentrations of IcdP present in retinal cells promotes degenerative changes comparable to the ones observed in age-related macular degeneration (AMD). Using human retinal cells simultaneously exposed to individually low-toxic doses of IcdP and HEV light wavelengths from solar simulator, we found that, in spite of oxidative stress generation, IcdP-HEV light toxic impact on cells is not a direct consequence of photosensitized oxidation reactions. Instead, their interaction results in loss of the tight coupling required between the two metabolic phases ensuring IcdP efficient detoxification. Indeed, IcdP/HEV co-exposure induces an over-activation of the aryl hydrocarbon receptor (AhR) signalling – dependent transcription of CYP1 genes and an accumulation of the cytochrome P450 monooxygenase CYP1A2 involved in phase I of metabolism. In addition, IcdP/HEV interaction is associated with a loss of nuclear factor erythroid-2 related factor-2 (Nrf2) and of Nrf2controled maintenance of glutathione S transferase (GST) proteins, responsible for phase II. Our data thus indicate a phase II hindered in response to co-exposure and insufficient to sustain the enhanced phase I induction. This is reflected by an accelerated endogenous reactive oxygen species (ROS) production and an increased accumulation of IcdP-related bulky DNA damage in retinal cells. Our work raises the prospect that lifestyle and environmental pollution may be significant modulators of HEV light toxicity in the retina.

Degradation of plastic nanoparticles exposed to UV light, toxicological consequences on human intestinal cell models

Maeva Boulée[1], Thierry Douki[1], Vérane Bard[1], Marie Carrière[1]

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Plastics, which are synthetic polymers containing chemical additives, are an indispensable material in our society because of their excellent properties (low cost, resistance, versatility). Their production has grown considerably and has resulted in significant environmental pollution due to inappropriate waste disposal management. During their passage through the environment, plastics are degraded by physical, chemical and biological agents, such as UV exposure or temperature, which lead to their fractionation into smaller particles, called micro and nanoplastics (MNPs). Unfortunately, currently available toxicity data on MNPs are insufficient to assess their overall impact, for example following ingestion.

The present study aimed at characterizing the impact of environmental conditions, i.e. exposure to UV light and high temperatures, on the physico-chemistry and toxicity of two plastic nanoparticles, polystyrene (PS) and polycaprolactone (PCL), chosen as models of non-biodegradable and biodegradable plastics, respectively. 100-1000 nm PS and PCL nanoparticles were artificially weathered in a Q-SUN test chamber, in conditions mimicking UV irradiation and temperature of a sunny day at noon in the equator region. Their transformation were characterized using dynamic light scattering, zeta potential characterization, FTIR and Raman spectroscopy, transmission electron microscopy as well as HPLC-MS/MS. Their toxicity towards human intestinal cells was analyzed using co-cultures of Caco-2 enterocytes and HT29-MTX mucus-secreting cells, representative of healthy people (wild type) or of people with susceptibility to Crohn's disease (Caco-2-NOD2^{1007fs}).

Our results show that physicochemical characteristics of PS and PCL NPs evolved during artificial weathering, especially the agglomeration state and shape. In addition, PCL NPs were shown to release some soluble low molecular weight substances while PS did not. Although PS and PCL NPs did accumulate inside both cell lines, no overt toxic effects of PS and PCL particles were observed. Cell viability, epithelial integrity, reactive oxygen species intracellular levels, inflammatory status and DNA integrity remained unchanged. These results are in agreement with the current literature, showing that plastic particles only show mild toxicity to intestinal cells, *in vitro*. The cell models and exposure conditions should be refined in order to highlight more subtle effects of these particles.

This work received funding by the Agence Nationale de la Recherche (ANR), under the grant ANR-21CE34-0028 (PLASTOX), from the Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES) under the grant EST-21-077 (EXaMINA), and from the Agence de l'environnement et de la maîtrise de l'énergie (ADEME).

Effects of CdSe/ZnS-COOH quantum dots on autofluorescence properties and proliferation of *Scenedesmus quadricauda* microalgae cells: dependence on growth medium composition

Agne Kalnaityte[1], Danguole Montvydiene[2], Emilija Januskaite[1], Zivile Jurgelene[2], Mindaugas Kazlauskas[2], Nijole Kazlauskiene[2] and Saulius Bagdonas[1]

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2 Nature of Research Centre, Institute of Ecology, Akademijos st. 2, LT-08412, Vilnius, Lithuania

Scenedesmus sp. are one of the most popular green freshwater microalgae species used in toxicity studies, because they often serve as model organisms for more complex plants in the studies to assess the toxic effects of substances on ecosystems and global environmental processes. These days, various pollutants can be detected in natural waters, including nanoparticles (NPs). Optical methods can be useful for monitoring their fate, since the change in the state of quantum dots (QDs) under the influence of various environmental factors (solvent, light, biological materials) is sensitively reflected in changes in the photoluminescence (PL) intensity and a spectral shift of the PL band. Moreover, the studies of autofluorescence of algae have revealed the ability of core QDs to retard the photoadaptation of wild type algae under naturally varying illumination conditions [1]. In this study, after evaluating the photostability of negatively charged hydrophilic CdSe/ZnS-COOH QDs in different aqueous media under light exposure, methods of optical spectroscopy and microscopy were applied to investigate the effects of these QDs on the growth, photosynthesis activity and population structure of green *Sc. quadricauda* microalgae in various water environments such as Balsys Lake water, deep well water, and MWC algal growth medium. The different composition of the growth medium has influence on both algae cells and QDs. Not only it can affect the supply of nutrients to cells, moreover, the higher ionic strength in the growth media than in distilled water promotes aggregation of the QDs, which does not suppress their contacts with algae cells and leads to unpredictable interactions that have not been previously studied. Algae play a key role in the aquatic food chain; therefore, the present study provides a useful information on how nanomaterials can affect the bioenvironment and how the complex environmental conditions can affect NPs.

This work was funded by the Research Council of Lithuania, Project No. S-MIP-20-22.

Reference: [1] A. Kalnaitytė, S. Bagdonas, Light-mediated effects of CdTe-MSA quantum dots on the autofluorescence of freshwater green microalgae: Spectroscopic studies, Journal of Photochemistry and Photobiology B: Biology, vol. 199 (2019)

Photopollution on dermal fibroblasts: a synergistic toxicity effect

Eloïse Larnac[1], Alicia Montoni[1], Sébastien Méthot[1], Laurent Marrot[2], Patrick J Rochette[1,3]

1 Centre de recherche du CHU de Québec – Université Laval, Axe médecine régénératrice, Hôpital du SaintSacrement
2 Advanced Research, L'OREAL Research & Innovation, Aulnay-sous-Bois, France 3 Département d'ophtalmologie, Faculté de Médecine, U Laval

Problematic: Pollution and ultraviolets A (UVA) are two environmental factors implicated in premature skin aging[1,2]. When absorbed by chromophores, UVA lead to the formation of reactive oxygen species (ROS), which can attack lipids, DNA and proteins[3]. Benzo[a]pyrene (BaP), an ubiquitous lipophilic atmospheric pollutant is able to absorb UVA[4]. Moreover, BaP has been shown to have a strong affinity for mitochondria, an organelle known to play an important role in premature skin aging[5,6]. Thus, our **hypothesis** is that co-exposure of BaP and UVA leads to skin fibroblasts alteration, which accelerates skin aging. We aimed to (1) demonstrate the toxic synergy of BaP/UVA on skin fibroblasts (2) and to decipher the effect of this synergistic toxicity on mitochondria.

Method: Dermal fibroblasts or isolated mitochondria were treated with BaP and UVA. Cell viability was evaluated using colorimetric assay. ROS production as well as lipid peroxidation were measured using fluorescent probes. DNA adducts formation was measured using a novel qPCR method. The impact of UVA/BaP on mitochondria was evaluated by microscopic techniques and western blot.

Results: The BaP/UVA synergy induces cell death along with an increase of ROS formation, lipid peroxidation and DNA adducts. On isolated mitochondria, the co-exposure BaP/UVA disturbs mitochondrial membranes and increases lipid peroxidation. In both models, lipid peroxidation can be prevented using a lipophilic antioxidant but not its hydrophilic counterpart.

Conclusion: The BaP/UVA synergy sensitizes dermal fibroblasts through oxidative stress, which lead to lipid peroxidation and the formation of DNA adducts. Lipid peroxidation can be prevented using a lipophilic antioxidant. We also found that mitochondria are highly sensitive to the toxic synergy BaP/UVA, suggesting the implication of this organelle in photo-pollution-induced premature skin aging. Our future works will focus on investigating the implication of photo-pollution on skin integrity.

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Parallel symposia

Thursday August 31

Afternoon

Antimicrobial PDT for environmental applications

Invited speakers:

IL106 Adelaide Almeida (Aveiro, Portugal)

Revisiting Methylene Blue: recent advances towards pathogenic microorganisms

IL107 Santi Nonell Marrugat (Barcelona, Spain)

The photochemical basis of blue-light photodynamic inactivation of bacteria

IL108 Reza Ghiladi (North Carolina, USA)

Photodynamic and Photothermal Materials for Infection Prevention in Healthcare Environments

IL019 Kristjan Plaetzer (Salzburg, Austria)

Agricultural applications of Photodynamic Inactivation

Oral communications:

OC116 Aleksandra Nyga: Multifunctionality of photoactive compounds.

OC117 Giacomo Insero: UVC Light Barriers as a New Tool in the Prevention of Airborne Viral and Bacterial Epidemic Spread

OC118 Gilberto Ú. L. Braga: Successive applications of photodynamic treatment against a plant-pathogenic fungus result in increased cell tolerance and carotenoid accumulation

OC119 Linda Jernej: Photodynamic Inactivation of Penicillium digitatum: a green post-harvest technology

Revisiting Methylene Blue: recent advances towards pathogenic microorganisms.

Adelaide Almeida[a], Cátia Vieira[a], Maria Bartolomeu[a], Daniela Silva[a] Ana Gomes[a], Cristina Dias[b], Leandro Lourenço[b], Carlos Monteiro[b], Nuno M. M. Moura[b], Maria G.P.M.S Neves[b], Maria A.F. Faustino[b]

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Antimicrobial photodynamic therapy (aPDT) is recognized to be an important strategy to deal with antimicrobial resistance, *which is considered nowadays a* serious *threat to global* public *health.* Although aPDT seems to be a very promising alternative to conventional antimicrobials, there are still important hurdles that currently prevent its implementation in practice. An important aspect to be considered is related with the synthetic access to new cost-effective and efficient photosensitizers (PSs). In this context, the exploitation of the widely available methylene blue (MB) can be a viable and economical option. This dye use is already well established for diagnosis, therapy and also in blood decontamination, which is considered safe. MB presents high binding affinity to multiple microbial targets, that together with its hydrophilicity, rendering it a straightforward PS for aPDT. In this communication, the efficiency of MB in the photoinactivation of bacteria, fungi and viruses will be discussed, first in phosphate buffer solution (PBS) (*in vitro*) and after in different settings, relevant to clinical and environmental areas: blood, urine, kiwifruit pollen and wastewater (*ex vivo*). The results show that MB was effective in the photoinactivation of bacteria and viruses in less complex matrices, but not so effective against fungi, when compared with porphyrin derivatives, such as the tetracationic TMPyP. Although the effectiveness of MB decreases with the increase in samples complexity, our studies demonstrate that its photodynamic efficiency can be improved with KI used as adjuvant. The effective reduction of different microorganisms with MB, associated to its safety, provided promising indications towards its safe use, namely in scenarios in which neither commercial PSs nor in-house preparation are available or when large areas need to be treated, as in some environmental applications.

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The photochemical basis of blue-light photodynamic inactivation of bacteria

Santi Nonell,[1,2] Luciano Dibona-Villanueva,[1,2] Roger Bresolí-Obach,[1,2] Oscar Gulias,[1,2] Light4Lungs group[2]

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Recent concerns over antimicrobial resistance have prompted increased attention to photodynamic therapy (PDT) as an approach to control bacterial infections. Antimicrobial resistance of *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria increases morbidity and mortality in patients with cystic fibrosis and hospital-acquired lung infections [1]. Blue-light therapy takes advantage of bacterial endogenous photosensitisers to eliminate pathogenic bacteria by an intracellular photodynamic effect [2]. This work aims at understanding the photochemical basis of blue-light antimicrobial therapy as a means to develop strategies towards improved therapeutic outcomes. Our results show that *S. aureus* and *P. aeruginosa* generate intracellular singlet oxygen upon exposure to blue light and that singlet oxygen is the main species responsible for bacterial photoinactivation. Therefore, pre-incubation of the bacteria with 5-ALA and potassium iodide is an effective strategy to enhance blue-light photoinactivation of *S. aureus* and *P. aeruginosa*.

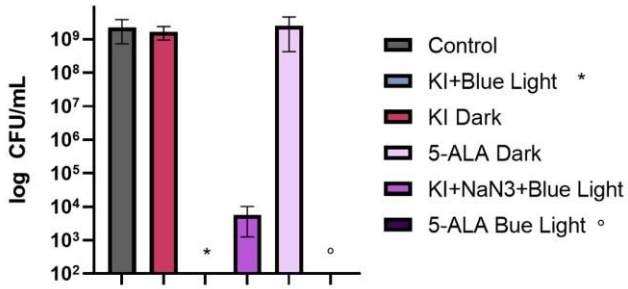

Figure 1. Blue light photoinactivation of S. aureus in the presence of singlet oxygen enhancers (potassium iodide, and 5-ALA) and quenchers (sodium azide)

Acknowledgment: This work was supported by the European Union's Horizon 2020 research and innovation programme under grant agreement nº 863102. www.light4lungs.eu

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Photodynamic and Photothermal Materials for Infection Prevention in Healthcare Environments

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Efforts to control hospital acquired infections (HAIs) have been hampered by the emergence of drugresistant pathogens, necessitating the pursuit of advanced functional materials that are capable of the self-disinfection of such microbes in hospital environments. To that end, we have explored two approaches for pathogen reduction: antimicrobial photodynamic inactivation (aPDI) and antimicrobial photothermal inactivation (aPTI). *In vitro* aPDI studies were performed against bacteria and viruses employing photosensitizer-embedded or conjugated nanofibrillated cellulose, polyacrylonitrile or nylon nanofibers, dual-dyed wool/acrylic blended fibers, olefinic block copolymers, and spray coatings. For natural polymer scaffolds [1], cellulose-porphyrin conjugates (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%). 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; ii) using melt-pressing [2], 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; and iii) we have explored photodynamic coatings on polymer microfibers for pathogen inactivation [3,4], and have demonstrated population reductions of >99.9999 and 99.6% for *S. aureus* and antibiotic-resistant *E. coli*, respectively, after exposure to visible light for 1 h. In response to the current COVID-19 pandemic, we also confirmed that these coated fibers can inactivate a human common cold coronavirus serving as a surrogate for the SARS-CoV-2 virus. As an alternative strategy, we have embedded the photothermal photosensitizer molybdenum disulfide (MoS_2) into bacterial cellulose (BC) by a facile *in situ* growth followed by a dipcoating process to add a chitosan layer (BC/MoS_2-CS) [5]. When compared with the BC/MoS_2 membrane, which itself showed a 95% reduction of *S. aureus* and 99.9% inactivation of *E. coli*, the addition of chitosan led to a synergistic effect, improving inactivation to 99.998% against *S. aureus* and 99.988% versus *E. coli*. More recently, we have developed dual-coated fabrics employing photosensitizerluminous powder using silk-screen printing, and demonstrated that this strategy is able to produce antimicrobial materials that function even when illumination ceases. Together, these results demonstrate that such 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.

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Agricultural applications of Photodynamic Inactivation

Christoph Hamminger[1], Annette Wimmer[1], Andreas Fellner[1], Linda Jernej[1], Mariana Vasconcelos[1], Jun Liu[2], Michael Fefer[2] and Kristjan Plaetzer[1]

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Access to healthy, safe and sufficient food represents a fundamental human right. Bacteria and fungi trouble growers by inducing plant diseases, by limiting the shelf life of products or, if human pathogens are involved, by contaminating food. Application of pesticides including antibiotics in agriculture has a negative impact on the environment and fires development of antimicrobial resistance. Photodynamic Inactivation (PDI) is a powerful tool to kill microorganisms[1]. The aim of this study is to demonstrate that PDI is applicable to fight microbial pathogens at various stages of the agricultural food production chain. Formulations of sodium-magnesium-chlorophyllin (Chl, approved as EU food additive E140) serve as economic and eco-friendly photoactive substances and are tested to fight bacterial and fungal plant pathogens as well as their application to improve *post* harvest microbial food safety. Depending on the application, concentrations of 100 µM to 2 mM of Chl followed by illumination with blue LED light or sunlight in the rage of about 50 to 100 J/cm^2 induces a clear antibacterial effect against plant pathogens such as *Erwinia amylovora* or *Botrytis cinerea*, irrespective of their resistance against conventional treatment[2] without harming host plants, as proven using *Fragaria vesca* as model system[3]. The same experimental approach is applicable for fighting mycelia and spores of *Penicilium digitatum*. Using an orange peel model, we demonstrate that PDI also helps to minimize food spoilage by *P. digitatum* and therefore has the potential to improve shelf life of fruits. Furthermore, PDI based on 100 µM Chl reduces the load of *Listeria innocua* on mung bean and radish seeds by 99.9 % (radiant exposure 56.4 J/cm² and 28.2 J/cm², respectively), and of buckwheat seeds by <90% (28.2 J/cm²) without affecting the germination rate. Photodecontamination of seeds partially propagates to germinated sprouts: the bacterial load on mung bean sprouts is reduced by more than 99.9% after phototreatment of seeds with 100 µM Chl[4]. As conclusion, PDI based on formulations of Chl can be applied in agriculture to fight plant diseases, for *post* harvest treatment of crops and to improve microbial safety of food.

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Multifunctionality of photoactive compounds

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For a few years, we are able to experience the dynamic development of optoelectronic technologies such as the most known OLEDs and OPVs. Consequently, we are witnessing how the quality of modern life is improving day by day. On the other hand, the development of modern optoelectronic technologies is often inhibited by the large impact of non-radiative transitions on the efficiency of photoactive devices. The non-radiative losses can be connected among all with a vibrationinduced quenching, a concentration quenching, other energy transfers, and last but not least, oxygen quenching. In the rush for better and more efficient optoelectronic systems, non-obvious applications of examined organic compounds are often missing. Such an alternative may be the singlet oxygen photogeneration by photoactive, conjugated molecules typically designed for optoelectronic applications. Going further, such molecules, capable of achieving high efficiency of the $^1O_2$ photogeneration process, can positively influence the development of medical applications, by taking advantage of the photosensitizing tendency, in various types of therapeutic treatment, including PDI (Photodynamic Inactivation).

The presented results focus on the overview of typical optoelectronic systems capable of photogeneration of singlet oxygen [1]–[3]. Results concern conjugated organic molecules such as fullerene and porphyrin derivatives, typically applied as photoactive layers in organic photovoltaic devices. The photoactive compounds were deposited with various techniques such as spin-coating, electropolymerization and chemical grafting. The presented research highlights the multifunctionality of photoactive materials and reveals great research potential at the border of the fields of photobiology and organic optoelectronics.

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UVC Light Barriers as a New Tool in the Prevention of Airborne Viral and Bacterial Epidemic Spread

Insero G.[1], Garzella F. [1], Fusi F. [1], Toci G. [2], Patrizi B. [2], Pollini S. [3], Baccani I. [3], Quaranta P. [4], Pistello M. [4], Rossi F. [5], Romano G.[1]

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5 Institute of Applied Physics "Nello Carrara", National Research Council (IFAC-CNR), Florence, Italy The UVC radiation (200-280nm) is the most effective wavelength for the inactivation of viruses and bacteria [1, 2], corresponding to the DNA and RNA absorption peaks, but may also be mutagenic. However, the recent Covid-19 pandemic and the well-known antimicrobial resistance issue has pushed for a re-evaluation of UVC light sources around 220 nm in presence of human beings: the 220-nm light penetration in biological media is limited to a few micrometer by the superficial *stratum corneum* of the skin, thus preventing light absorption by the underlying live tissue, together with a local minimum absorption by DNA/RNA at 230 nm [2, 3].

We present a 222-nm light barrier for the suppression of airborne viral and bacterial spread to be used in situations with constrained geometries (e.g. public transportation, offices, waiting rooms etc.) in presence of humans. The proper design of the light barrier is based on the sterilization efficacy study that we conduct using a 222-nm excimer KrCl mixture lamp providing 4.5mW/cm^2 in *in-vitro* culture of SARS-CoV-2 of Wuhan lineage and of 2 strains of *Staphylococcus aureus* and 2 strains of *Pseudomonas aeruginosa*, that we select as representatives of clinically relevant bacterial pathogens (wild-type reference strains and strains of clinical origin harboring relevant antibiotic resistance mechanisms, i.e. carbapenem and oxazolidinones resistance enzymes). The pathogens have been irradiated using the 222nm light at fluence ranging from 0 to about 35 mJ/cm^2. The photokilling efficacy was estimated by viable cell counts and by the Spearman-Karber method for bacteria and viruses, respectively. We demonstrated strong antiviral effect on SARS-CoV-2 at 7.5 mJ/cm^2 (4-log reduction of the viral titer) and strong antibacterial effect at around 15 mJ/cm^2 in all tested bacterial strains (3-log reduction). To fully demonstrate the capabilities of this innovative UVC-light barrier, it will be used to suppress airborne SARS-CoV-2 spread in an *in-vivo* experiment using mice. In the future, the presented light barrier can be used as a new tool for environment decontamination in presence of human being.

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Successive applications of photodynamic treatment against a plant-pathogenic fungus result in increased cell tolerance and carotenoid accumulation

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Antimicrobial photodynamic treatment (APDT) is a promising alternative to control both human- and plant-pathogenic fungi and many studies suggest that selecting tolerant strains is unlikely since APDT targets a myriad of cell components. *Colletotrichum abscissum* is an important plant-pathogenic fungus that causes post-bloom fruit drop in *Citrus* species worldwide. Control of the fungus is performed with conventional antifungal agents such as strobilurins and triazoles, which could lead to the selection of resistant strains. Here we investigated whether consecutively applying APDT on *C. abscissum* conidia could result in selection of resistant strains. Conidia of *C. abscissum* were treated with the photosensitizer new methylene blue N and red light. After the treatment, surviving conidia were allowed to develop into colonies and conidia from these colonies were collected, pooled, and used to perform the next round of APDT. After 60 cycles, we selected a population that displayed higher tolerance to APDT when compared to the initial strain. We also observed that the 60th-cycle population produced conidia with a stronger orange hue. We performed carotenoid extraction, characterization, and quantification with HPLC-DAD-MS/MS and observed that the selected fungi accumulated more carotenoids than the initial strain. Carotenoids are known scavengers of singlet oxygen, the main reactive species produced during APDT. An increased carotenoid content is a possible explanation for the higher tolerance to APDT observed for 60th-cycle population. Our results indicate that the sequential application of APDT can select fungi with increased tolerance to the treatment.

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Photodynamic Inactivation of *Penicillium digitatum*: a green post-harvest technology

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During post-harvest storage, citrus fruits may be spoiled with several pathogens. One of them is the fungus *Penicillium digitatum,* causing green mould. Especially in subtropical and arid climate zones, the disease is to blame for 90% of total crop losses in citriculture, resulting in great economic losses for farmers, distributors and grocers[1]. Existing post-harvest treatments of oranges include chemical (H_2O_2), physical (radiation) and antimicrobial (fungicides) treatments[2]. In this study, Photodynamic Inactivation (PDI) is introduced as effective, cheap, and eco-friendly approach to combat *P. digitatum* in the post-harvest application. To provide eco-friendliness, sodium-magnesium-chlorophyllin (Chl, approved as EU food additive E140[3]) serves as photosensitizer and is combined with different surfactant molecules and a chelator[4]. Illumination of all samples was done using an LED array with 395 nm. The technique was tested on various model systems including liquid spore culture, mycelial spheres and orange peel plugs. In 5 out of 8 samples, each containing 500 µL of a 10^6 spores/mL suspension and treated with 0.015% Chl and 106 J/cm^2, no hyphae growth was observed after a 7-day incubation. Treating mycelial spheres using the same concentration of Chl and the same radiant exposure achieved a total kill (100 %). Seventy percent of orange peel plugs infected with 10 µL of a 10^6 spores/mL suspension of *P. digitatum* were free from fungal spoilage after PDI treatment with 0.0325% Chl and 106 J/cm^2 radiant exposure. This work clearly demonstrates that PDI based on chlorophyllin can serve an effective, environment friendly and safe method to decontaminate citrus from *P. digitatum.* Post-harvest treatments based on PDI can lead to increased yields in citriculture and will decrease economic losses for farmers, distributors, and grocers.

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Update on physiological effect of Sunbeds

Invited speakers:

IL110 Olivier Merckel (Maison-Alfort, France)

Public Health regulations of sunbeds

IL111 Astrid Coste (Lyon, France)

Melanoma epidemics in Iceland and Faroe islands

IL112 Rudiger Greinert (Buxtehude, Germany)

Sunbed use. Who, when, where ?

IL113 Thierry Douki (Grenoble, France) Contribution of UVA to the effects of sunbeds

Oral communications:

OC120 Manuel Alejandro Herrera Lopez: effects of UVA and visible light on the mitochondrial activity of keratinocytes

OC121 Ewan Eadie: A two-stage preliminary investigation of the visual and histological changes to healthy volunteer skin from single and repetitive exposure to virucidal Krypton-Chloride (KrCl*) Far-UVC-emitting excimer lamps

Public Health regulations of sunbeds

Olivier Merckel

Anses, Maisons-Alfort, France

Since 26 January 2016, the sale of tanning equipment to private individuals has been banned in France. This public health measure was introduced 17 years after artificial ultraviolet radiation was classified as carcinogenic to humans by the International Agency for Research on Cancer. However, in 2023, this ban is still only theoretical. The draft decree that was supposed to make this decision applicable, through a complex mechanism involving European standards and regulations, has not yet been adopted. This missed opportunity is the latest episode in a slow regulatory evolution that is out of step with scientific knowledge and health risk assessment.

Indeed, since its creation in 2005, Anses has recommended that people avoid exposure to artificial UV radiations. The Agency subsequently published several opinions recommending that, in order to protect human health, the commercial use and sale of equipment delivering artificial UV for aesthetic purposes should be discontinued (2012, 2014, 2018). Taking a less restrictive approach, French regulations have strengthened consumer information (2013), particularly on the health risks involved by artificial UV exposure, recommendations for use and photosensitizing effects. In 2016, a new law prohibited certain commercial practices, such as promoting free sessions, promotional offers, or making people believe that exposure to artificial UV radiation has a beneficial effect on health.

However, checks on compliance with these obligations in recent years (2017 to 2019) have shown that more than half of the establishments concerned were not complying with the regulations.

It is likely that it will only be through concerted and coordinated actions by several Member States at European level that national regulations will at last fully protect human health.

Artificial ultraviolet radiation and skin melanoma in Iceland and the Faroe Islands

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(250 word/250)

Background: A drastic increase in the incidence of skin melanoma following substantial exposure to sunbeds has been document in Iceland from 1995 to 2010 (Hery et al, AJE, 2010). High sunbed exposure has also been reported in the Faroe Islands.

Objectives: To document sunbed usage patterns and analyse melanoma incidence and mortality trends in Iceland and the Faroe Islands.

Methods: 5-year moving averages of age standardized incidence rates from 1960 to 2020 were computed using the NORDCAN data. Comparisons with other Nordic countries were made. Updated data on sunbed use were searched.

Results: The sunbed fashion started around 1980. In Iceland, 25% of subjects aged 12-15 years in 2004 used sunbeds in the last year. In the Faroe Islands, 44% of subjects born in 1963-77, and 67% of subjects born in 1978-92 used sunbeds before 18 years of age. After 1980, 5 to 10-fold increases in melanoma incidence occurred, mainly among females, with peak incidence rates of same magnitude in Iceland (2003), and the Faroe Islands (2006). Incidence rates levelled off after 2010, when restriction policies were introduced, but remained 2 to 4 times higher than before 1980. After merge of data from both countries, a 4.8-fold increase in numbers of melanoma deaths has been observed since 1985-89 (for 1.8-fold increase in Norway). Epidemiological observations cannot be explained by changes in dermatology services.

Conclusion: Despite small numbers of cases, comparable episodes of melanoma epidemic have affected Iceland and the Faroe Islands following massive exposure to sunbeds starting at young ages.

Sunbed use: who, when, where ?

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Tanning bed use has been classified as carcinogenic to humans by the IARC in 2009. Nonetheless, individuals in Western industrialized countries still use tanning beds. For Europe, data collected from 2009 to 2014 showed that the prevalence of having ever used a tinning bed was 10.6% (Suppa et al. 2019). Detailed representative data from Germany on 14- to 45-year-olds showed a decrease in current tanning bed use (i.e., use within the last 12 months) from 2015 (11.0%) to 2018 (8.8%; Diehl et al. 2019). In 2019, the prevalence in 16-to 65-year-old German citizens was 7.5%. There was no significant difference between women and men. Those aged 46 to 55 years, those with immigrant background, those with higher education, and those being employed were more likely to use tanning beds than their counterparts (Diehl et al. 2022a). Trend data from Germany also show a change in place of tanning bed use. While in 2015 76.5% of current users used a tanning bed in a tanning salon, the proportion decreased to 57.0% in 2019 (Diehl et al. 2022b). At the same time, the proportion of users in spa, fitness, and beauty facilities increased from 20.6% to 39.1% (Diehl et al. 2022b). Although it is encouraging that the prevalence of tanning bed use is decreasing, it is concerning that its use is shifting to settings that are actually for relaxation (spa and beauty facilities) and health and athletic fitness (fitness facilities). Results are discussed in comparison to European and worldwide develöpments.

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Contribution of UVA to the effects of sunbeds

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A characteristic of UV lamps found in sunbeds is that their spectrum is more intense and richer in UVA (320-400 nm) than natural sunlight. The latter wavelength range has long been considered as negligible compared to more energetic UVB (280-320 nm). However, a growing amount of information is now available showing that UVA exhibits deleterious effects in human skin.

A well-documented phenomenon is photoaging associated with oxidative stress that degrades components of the dermal extracellular matrix. Release of oxidizing species by photosensitized processes is also involved in many processes such as lipid peroxidation and protein degradation. DNA is another target of UVA-mediated oxidative stress with formation of oxidized bases and strand breaks. Interestingly, it is now well established that UVA is weakly absorbed by DNA where it induces the formation of pyrimidine dimers like UVB, although with a lower efficiency. UVA-induced formation of pyrimidine dimers can also occur in the dark through oxidative processes like the formation of melamine peroxides. UVA exhibits also systemic properties such as immunosuppression.

Interestingly, UVA can act in synergy with UVB. For example, it is now well established that UVA, likely through protein oxidation, degrades DNA repair enzymes and hampers the removal of UVB-induced photoproducts. The combination of these different mechanisms would be synergic in terms of mutagenesis. These observations strongly support a possible role of UVA in the increased frequency of melanoma in sunbed users.

Altogether, it appears that UVA is not a harmless physical agent. It is actually classified as carcinogenic to humans. Since it is now well documented than the pigmentation induced by artificial sunlight does not afford skin photoprotection, use of sunbeds results in a voluntary exposure to a damaging agent without health benefit.

Effects of UVA and Visible Light on the Mitochondrial Activity of Keratinocytes

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Photons in the UVA and visible ranges of the sun spectra penetrate deep into the skin, bringing unknown metabolic challenges to cells in the epidermis and dermis. We propose to study the *in vitro* activity of keratinocyte mitochondria by analyzing how mitochondrial respiration is affected by exposure to different ranges and doses of UVA (λ_{Max}= 365 nm) and visible light (VL, blue-λ_{Max}= 450 nm, green-λ_{Max}= 522 nm and red-λ_{Max}= 657 nm), using real-time cell metabolic flux analyzer Seahorse XF, which measures oxygen consumption rates (OCR) (Fig 1A). Basal OCR is obtained with the cell without any intervention, corresponding to the oxygen used for the entire cell. The addition of oligomycin (an ATP synthase inhibitor), allows evaluation of OCR associated with ATP synthesis and the proton leak. Maximal OCR is obtained by an uncoupling agent such as CCCP. The final step of a typical Seahorse trace allows evaluation of nonmitochondrial OCR in the presence of rotenone and antimycin A (electron chain inhibitors). UVA and bluelight exposure cause significant reductions in the basal, maximal respiration and OCR associated to ATP synthesis, with minimal changes in the proton leak. Green light does not show any effect on mitochondrial respiration, while red light, surprisingly, substantially boosts almost all respiration rates (Fig 1B). The trend observed from UVA /Blue (inhibition), passing to the neutral responses of green, to the activation of red, suggests the presence of two opposite effectors controlling the responses of mitochondria to light stimulus. Our findings provide a better understanding of the interactions between light and human skin, which will be important for the development of novel strategies in sun protection and photomedicine.

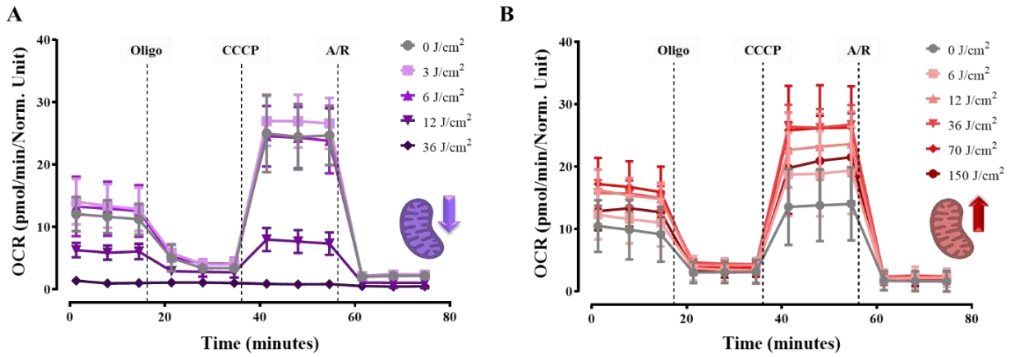

Figure 1. *Evaluation of mitochondrial respiration by real-time cell metabolic analysis for keratinocytes cells irradiated at 365 nm (A) and 657 nm (B). Mitochondrial oxygen consumption was modulated under basal conditions and in the presence of ATP synthase inhibitor oligomycin (Oligo), uncoupler CCCP, and respiratory inhibitors antimycin A plus rotenone (A/R).*

A two-stage preliminary investigation of the visual and histological changes to healthy volunteer skin from single and repetitive exposure to virucidal Krypton-Chloride (KrCl*) Far-UVC-emitting excimer lamps

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Krypton-Chloride (KrCl*) Far-UVC-emitting excimer lamps can quickly and efficiently inactivate airborne pathogens [1]. In-vitro and in-silico experiments suggest the emitted Far-UVC does not penetrate far into human tissue, resulting in proposals to irradiate populated rooms for the reduction in transmission of airborne diseases [2]. We wished to study, in-vivo, the visual and histological changes in skin with single or repeated exposures to Far-UVC. Twenty volunteers were recruited into this two-stage study, with eight in the single-exposure-dose-escalation Stage 1 and 12 in the repeated exposure Stage 2. No visual changes were observed in the skin for single exposures up to 3,000 $mJcm^{-2}$ or at sites repeatedly exposed to 1,500 $mJcm^{-2}$. There was also no significant increase in DNA damage (as assessed by cyclobutane pyrimidine dimers and double-strand breaks) when compared to non-irradiated skin, which offers some reassurance regarding safety, although this requires further evaluation. Skin reflectance measurement showed changes in b* (CIE LAB colour space), which represented a shift towards yellow colouration. These results conclude that Far-UVC has limited penetration into the top layer of skin as it is highly absorbed in the Stratum Corneum (SC). We hypothesize that this causes chemical changes within the SC, the nature and significance of which is currently unknown.

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Photofunctional proteins and peptides

Invited speakers:

IL114 Heikki Takala and Cornelia Böhm (Jyväskylä, Finland)

Function and application of a model bacterial phytochrome

IL115 Michael Westberg (Aarhus, Denmark)

Photoswitchable Binders for Control of Endogenous Proteins

IL116 Andreas Möglich, (Bayreuth, Germany)

Light & Temperature Reception by Plant Phytochromes

IL117 John Kennis (Amsterdam, The Netherlands)

Unusual isomerization dynamics in red-absorbing microbial rhodopsins

Oral communications:

OC122 Cédric Mittelheisser: Mechanism of a novel Reversibly Switchable Fluorescent Bacteriophytochrome revealed by time-resolved optical spectroscopy

OC123 Saeed Shareef: Building excitons in chromophore-proteins for light harvesting applications

OC124 Ursula Vide: Naturally occurring ON-OFF switches: Blue light-regulated LOV-diguanylate cyclases

OC125 Katharina Spies: Active Site Structure and Absorption Spectrum of the Channel rhodopsin Chrimson: Wild Type and Mutants

Function and application of a model bacterial phytochrome

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Phytochrome photoreceptors sense red and far-red light and cycle between the spectroscopically, structurally, and functionally distinct Pr and Pfr states. Upon light activation, the structural changes of the photosensory module of phytochrome are relayed to the enzymatic output module. Bacterial phytochromes commonly belong to two-component systems that transmit environmental stimuli to a response regulator protein through output histidine kinase (HK) activity.

We have revealed with cryo-EM that apparently minor structural changes in the photosensory module of the paradigm phytochrome from *Deinococcus radiodurans* (*Dr*BphP) cause a large change in its output HK module [1]. Unlike most HKs, *Dr*BphP exclusively acts as a phosphatase [2]. Its photosensory module, however, can control HK activity of homologous receptors. Inspired by this finding we have generated new optogenetic pREDusk and pREDawn tools, which enable the control of bacterial gene expression with red light [3]. Recently, we have modified the phytochrome component of these tools for reversed activity and improved red light sensitivity. We have also shown that the HK activity in phytochromes can be reversed with rational linker helix modifications [4].

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Photoswitchable Binders for Control of Endogenous Proteins

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Keywords: Optogenetics, Photodissociable Fluorescent Proteins, Protein Binders, DARPins

Abstract:

General methods for controlling specific endogenous proteins with high spatiotemporal resolution would be broadly useful for probing protein function in living cells. Synthetic protein binders that inactive endogenous protein of interest can be obtained from immunoglobulin domains, designed ankyrin repeat proteins (DARPins), and other small protein scaffolds, but generalizable methods to control their binding activity are limited. Here, we report robust single-chain photoswitchable DARPins (psDARPins) for bidirectional optical control of endogenous proteins. We created topological variants of the DARPin scaffold by computer-aided design so insertion of photodissociable dimeric Dronpa (pdDronpa) domains results in occlusion of target-binding at baseline. Cyan light induces pdDronpa dissociation to expose the binding surface (paratope), while violet light restores pdDronpa dimerization and paratope caging. As the DARPin redesign leaves the paratope intact, the approach was easily applied to existing DARPins for a range of targets as demonstrated by relocalizing GFP-family proteins and inhibiting endogenous kinases with optical control. Adding to the modularity, we have recently designed new pdDronpa variants with improved caging performance and developed a photodissociable red fluorescent protein. These photoactive domains should allow for tuning of psDARPin binding affinities and multiplexing. In summary, single-chain psDARPins provides a generalizable strategy for precise spatiotemporal dissection of the functions of endogenous proteins, including those that lack direct pharmacological antagonists.

Light & Temperature Reception by Plant Phytochromes

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Phytochromes perceive red and far-red light to elicit vital adaptations of plants. Downstream physiological responses revolve around red-light-induced interactions with phytochrome-interacting factors (PIF). Phytochromes moonlight as thermoreceptors, owing to the pronounced temperature dependence of thermal reversion from the light-adapted Pfr to the dark-adapted Pr state. Here, we assess whether thermoreception may extend to the phytochrome:PIF interactions. While the association between Arabidopsis thaliana PhyB and several PIF variants moderately accelerates with temperature, the dissociation does more so, thus causing net destabilization of the phytochrome:PIF complex. Markedly different temperature profiles of PIF3 and PIF6 might underlie stratified temperature responses in plants. Accidentally, we identified a hitherto unappreciated photoreception mechanism under strong continuous light, where counterintuitively the extent of phytochrome:PIF complexation decreases with red-light intensity rather than increases. Mathematical modeling rationalizes this attenuation mechanism and ties it to rapid red-light-driven Pr↔Pfr interconversion and complex dissociation out of Pr. Varying phytochrome abundance, e.g., during diurnal and developmental cycles, and interaction dynamics, e.g., across different PIFs, modify nature and extent of attenuation, thus permitting light-response profiles more malleable than possible for the phytochrome Pr↔Pfr interconversion alone. Our data and analyses pinpoint a previously disregarded photoreception mechanism with profound implications for plant physiology and biotechnological applications.

Unusual isomerization dynamics in red-absorbing microbial rhodopsins

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Bestrhodopsins are a newly discovered class of light-regulated ion channels that consist of two rhodopsins in tandem fused with a bestrophin ion channel domain. Bestrhodopsin of the marina alga *Phaeocystis antarctica* binds all-trans retinal Schiff-base (RSB) that absorbs at 660 nm, conveying excellent potential for optogenetic applications. Red light illumination of the tandem domain results in a metastable green-absorbing state P540, which corresponds to an unusual 11-cis RSB isomer rather than the canonical 13-cis isomer. Transient absorption (TA) spectroscopy showed that a primary photoproduct P690 is formed 1 ps at about 10% quantum yield, which evolves to a secondary product P670 in 550 ps. Next, P670 establishes an equilibrium with P590 in 2 µs after which it evolves to the metastable P540 species in 42 µs. Femtosecond stimulated Raman spectroscopy (FSRS) showed that P690 corresponds to a mixture of 11-cis and 13-cis RSB isomers. Strikingly, P670 corresponds to 13-cis, while P590 has switched to 11-cis, which is finally stabilized in P540. Hence, extensive isomeric switching on the ground state potential energy surface (PES) occurs on the sub-ns to microsecond timescale before bestrhodopsin finally settles on a stable 11-cis photoproduct. FSRS further indicates that P690 and P670 correspond to unusually highly distorted polyene backbones of RSB, while P590 and P540 are more relaxed. We propose that the observed phenomenology relates to trapping of the early photoproducts P690 and P670 high up the ground-state PES after passing through conical intersections (CIs) that result in both 11-cis and 13-cis RSB populations, caused by steric clashes with amino acid side chains. Corotation of C11=C12 and C13=C14 double bonds and adjacent single bonds upon passing through the CIs and subsequent trapping results in a constricted conformational landscape on the ground state PES that allows thermal switching between 11-cis and 13-cis species of highly strained RSB chromophores. On the microsecond timescale, protein relaxation may take place that releases the strain on the RSB chromophore allowing it to finally evolve to a stable 11-cis isomeric configuration. Hence, we have observed isomeric switching of structural intermediates close to the conical intersection in a microbial rhodopsin.

Mechanism of a novel Reversibly Switchable Fluorescent Bacteriophytochrome revealed by time-resolved optical spectroscopy

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Super-resolution fluorescence microscopy faces a recent challenge in developing new Reversibly Switchable Fluorescent Proteins (RSFPs) that operate in the red/NIR domain. We developed a new neariR-RSFP engineered on Deinococcus radiodurans bacteriophytochrome (Dr-BphP). The use and optimization of this new protein requires understanding its photo-switching mechanism, specifically to identify the controlling species (fluorescence and switching quantum yields). Through UV-Vis spectroscopy and TR-SFX studies, it has been shown that the photo-switching of Dr-BphP involves several processes, including cis-trans isomerization of the biliverdin chromophore, deprotonation/protonation steps and structural changes in the protein. The photodynamics takes place on a range of time-scales, from femtoseconds to milliseconds, involving several excited states and intermediates.

We investigate the photo-dynamics of our new iR-RSFP using femtosecond-millisecond transient UVVis-NiR absorption spectroscopy and nanosecond emission experiments. Comparison of different proteins (a fluorescent one, the photo-switchable Dr-BphP and iR-RSFP) allow us to discriminate two ground states species that mainly relax by fluorescence in 600 ps or isomerization in 40 ps, latter one the precursor of P_{fr}. The new iRRSFP shows different millisecond ground states species. I will discuss here the details mechanism of this new fluorescent and switchable phytochrome, as well as remaining questions that may be resolved through future time-resolved structural studies, in light of the structural studies on Dr-BphP.

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Building excitons in chromophore-proteins for light harvesting applications

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In nature, photosynthesis begins with light-harvesting, where specialized pigment-protein complexes transform photons into electronic excitations (excitons) and deliver them into the reaction center which initiates charge separation. This energy and electron transfer mechanisms have always been a fascinating model to develop novel energy conversion systems. These photosynthetic complexes are composed by densely packed chromophores (chlorophylls and carotenoids) into various protein matrices. The efficiency of photosynthesis depends on both the structural and electronic properties of these complexes [1].
Here we are presenting a systematic approach to develop chromophore-protein assemblies for artificial photosynthesis. Zinc-pheophorbide-a and its derivatives are used to mimic the pigment functions. The protein matrix consists of *de novo* designed single-chain amino acid sequences so-called maquettes. They contain 4-α helix bundles connected via random coils [2]. This protein platform allows us to modify the chromophore´s location and, therefore, to control their electronic properties. The hydrophobicity of the chromophores and the axial ligation of Zinc with Histidines have been exploited for binding the of chromophore to the with proteins. In order to create excitonically coupled dimers with charge-transfer character, we have produced various proteins with four binding sites (two at the top and two at the bottom of the protein structure, see Figure 1) with the potential to house two chromophore dimers, as well as control proteins with two binding sites (one at the top and one at the bottom of the structure) not capable to accommodate chromophore dimers. The nanomolar binding affinity of chromophores with all protein designs was determined using absorption titrations. Additionally, Circular Dichroism spectroscopy has been employed to investigate excitonic interactions, and revealed that only the four histidine-containing maquettes exhibited conservative Cotton effects (that is, excitonically coupled dimers) whereas the remaining two histidine-containing maquettes did not display such effect. The presence of charge-transfer states has been probed by Stark spectroscopy. Two parameters can be obtained from the Stark spectra: the change in transition dipole moment (Δμ) which indicates the degree of charge separation, and the change in polarizability (Δα) indicates the sensitivity of an electronic transition to an electrostatic field. The analysis of Stark spectra shows that there are significant differences in the spectral features of excitonically coupled dimer complexes as compared to non-excitonic complexes. Ultrafast measurements employing femtosecond Pump-Probe and 2-Dimensional Electronic Spectroscopic (2DES) techniques were carried out to gain further insights into the exciton dynamics and coherences of these complexes.

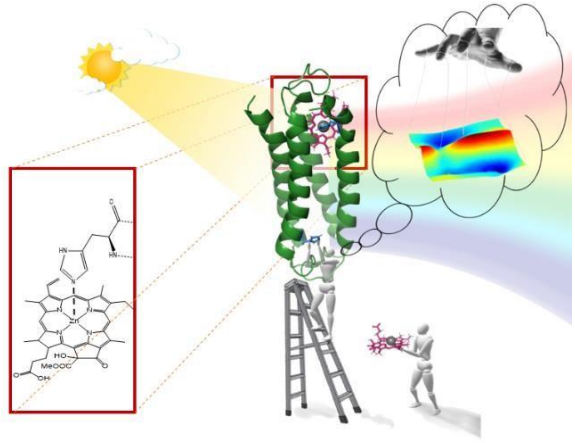

Figure 1. Schematic representation of building excitons and controlling it´s energy levels.

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Naturally occurring ON-OFF switches: Blue light-regulated LOV-diguanylate cyclases

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Photoreceptors sensing blue light were early on identified as key players in plant phototropism, which belong to the family of flavin-dependent light-oxygen-voltage (LOV) domains [1]. They also have a high potential in applied biosciences [2]. We investigated the molecular mechanisms of light regulation in LOV-activated diguanylate cyclase (LadC) with an exceptionally high fold change of enzyme activation over four orders of magnitude making it a switch-like system. We employed an integrative structural biology approach combining X-ray crystallography, solution scattering, computational methods and hydrogen-deuterium exchange coupled to mass spectrometry to reveal how this protein switch operates on a molecular level. The dark, inhibited conformation (**Fig.1A**) is characterized by a tight association of the sensory LOV and catalytic GGDEF domains in the dimeric assembly, which prevents the productive encounter of two GGDEF domains that would be required for catalysis. The efficient trapping of the effector domains is released upon structural rearrangements induced by blue light illumination (**Fig.1B**).

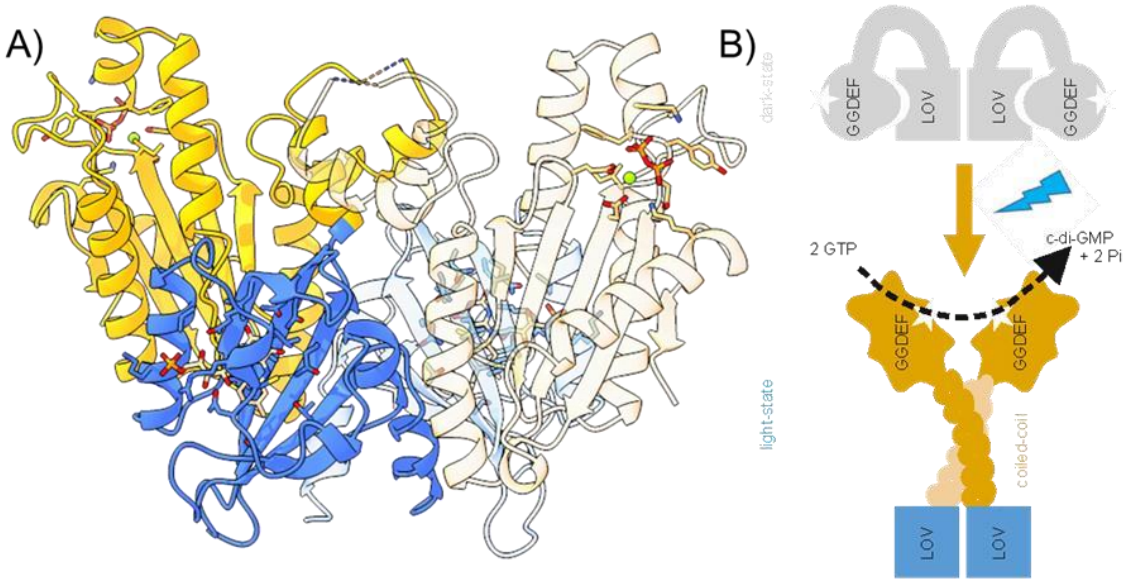

Figure 1: LOV-activated diguanylate cyclase switch mechanism; ***A)*** *Full-length crystal structure of dimeric darkadapted LadC with LOV domains in blue colors, linker helices and GGDEF domains in orange colors, and one protomer distinguished in transparency;* ***B)*** *Mechanistic model of the LadC switch, where blue-light exposure triggers rearrangements within the inhibited dimer, allowing the extension of the linker helices into a coiled-coil.*

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Active Site Structure and Absorption Spectrum of the Channelrhodopsin Chrimson – Wild Type and Mutants

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Due to its red-light activation and the associated low phototoxicity for cells, the channelrhodopsin Chrimson is widely used in optogenetic studies, e.g. for vision and hearing restoration. With our computational study based on the Chrimson crystal structure (PDB-ID: 5ZIH) [1], we aim to identify residues and structural conformations responsible for Chrimson's color tuning. We are specially interested in the structure of the active site, which includes the chromophore retinal with a positively charged Schiff base in the ground state structure and its counterions, the glutamate E165 and the aspartate D295.

We performed quantum mechanics/molecular mechanics (QM/MM) simulations of the Chrimson wild type and various mutants using the computationally favorable density functional tight binding method (DFTB3) as the QM method [2,3]. In addition, we calculated the excitation energies of a large ensemble of QM/MM trajectory snapshots to compare the absorption spectrum to other rhodopsins, such as channelrhodopsin 2 and bacteriorhodopsin. To obtain a valid computational model, we simulated multiple models with different protonation states of the counterions in the active site and the glutamates in the putative ion pathway, and compared the structural and spectroscopic properties.

We confirm the experimental studies [1,4,5] that the counterion E165 is protonated in Chrimson, which is an unique configuration among channelrhodopsins, and we also identify the protonation states of the glutamates in the central and outer gates of Chrimson. Our computed excitation energies are qualitatively in the correct range and the absorption spectra of structures with mutated residues in and near the active site also agree well with the experimental results. At least two stable configurations have been observed in the active site: the distance between the counterions is very small and the proton of the counterion E165 is shared by both, or the distance increases and a water molecule is present between the counterions. Furthermore, the orientation of the residues in the active site is highly interdependent, changing the hydrogen bonding network towards the retinal Schiff base and the counterion configuration. We were able to correlate the excitation energies with the structural motifs, and the absorption of Chrimson is shifted to red light when the shared proton motif is present.

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Biodiversity in light harvesting and regulation

Invited speakers:

IL118 Herbert van Amerongen (Wageningen, The Netherlands)

Exploring regulation of light harvesting in vivo with picosecond spectroscopy and microscopy

IL119 Tina Dominguez Martin (Cordoba, Spain)

Light-harvesting and photoprotection mechanism in cyanobacteria

IL120 Donatas Zigmantas (Lund, Sweden)

Mapping energy transfer in photosynthetic bacteria *in vivo*

IL121 Olga Chukhutsina (Amsterdam, The Netherlands)

Photoregulation of plants *in vivo*

Oral communications:

OC126 Justyna Łabuz: A large-scale study of chloroplast movements and dark positioning in plants from different light environments

OC127 Alexander Ruban: Hydrophobic mismatch: the missing link in the regulation of photosynthetic lightharvesting?

OC128 Sam Wilson: Isolation and analysis of the photoprotective locus in plants using the styrene-maleic acid copolymer

Exploring regulation of light harvesting *in vivo* with picosecond spectroscopy and microscopy

Herbert van Amerongen[1,2], Cleo Bagchus[1], Ludovico Caracciolo[1], Jeremy Harbinson[1], Lennart A. I. Ramakers[1], Dana Verhoeven[1], Emilie Wientjes[1].

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In our laboratory we study (amongst others) light harvesting and its regulation in photosynthetic (micro)organisms and plant leaves. We obtain both structural and functional information with the use of picosecond fluorescence spectroscopy and microscopy in combination with pulse-amplitude-modulated (PAM) fluorometry. In this presentation I will focus on some recent results that we obtained on plant leaves, regarding variation in antenna size, non-photochemical quenching (NPQ), state transitions and photoinhibition.

A few remarkable findings will be presented:

1) NPQ consists of at least of 3 different phases and one of them is partially replaced by another one during the build-up of NPQ.

2) After full activation of NPQ, part of NPQ can switch on/off instantaneously upon closing/opening of the reaction centers of photosystem II.

3) Upon state 1 to state 2 transition a significant fraction of the major light-harvesting complex II (LHCII) moves from the grana to the stroma lamellae, while the structure of the grana remains apparently unaltered.

4) During photoinhibition, the average fluorescence lifetime (and the fluorescence yield) decreases both in the grana and stroma lamellae.

I will end with a comparison of the mechanisms of NPQ and state transitions in plants, green algae, a variety of cyanobacteria and diatoms, showing a large biodiversity but always based on two basic mechanisms.

Mapping energy transfer in photosynthetic bacteria in vivo

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2 Institute of Physical and Theoretical Chemistry, University of Würzburg, Germany

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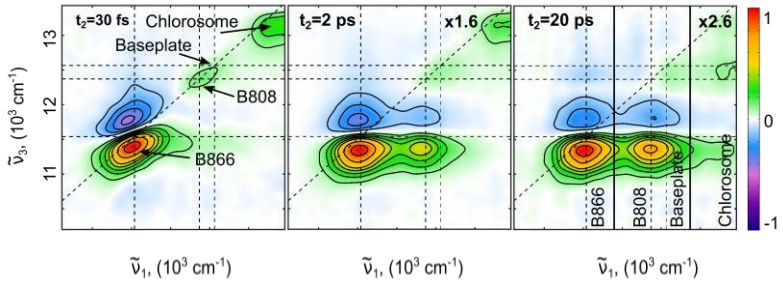
4 School of Biochemistry, University of Bristol, UK

The remarkable efficiency of solar energy collection in photosynthetic bacteria is assured by optimized arrangement of chromophores in light-harvesting complexes and by robust connectivity between complexes comprising photosynthetic units. While significant progress has been made during recent decades in understanding the energy transfer dynamics within isolated complexes, information about their functional connectivity in intact cells is still sparse or at least incomplete. A serious challenge for optical studies of energy transfer in intact photosynthetic systems is posed by the great complexity of photosynthetic units and strong light scattering, characteristic for suspensions of photosynthetic cells. To map and characterize light-harvesting processes in intact photosynthetic bacterial cells we use scatterresistant two-dimensional electronic spectroscopy (2DES) [1,2]. Here we present physiological temperature measurements of intact cells of the thermophilic green non-sulfur bacterium *Chloroflexus aurantiacus* and purple bacterium *Rhodobacter sphaeroides*. The two studied photosynthetic bacteria feature different architecture of photosynthetic units. We were able to fully map energy transfer processes between the light harvesting complexes and down to the reaction centers, with ensuing charge separation. As a distinct signature of the last step we observe clear transient electrochromic shift signal of one of the reaction center absorption bands. Then we apply global fitting procedure to characterize dynamics of all energy transfer channels in photosynthetic machinery of purple bacteria cells.



2DES spectra of Cfx. aurantiacus cells at room temperature

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Photoregulation of plants in vivo

Volha U. Chukhutsina

Vrije Universiteit Amsterdam, The Netherlands

Photosynthesis starts when a pigment in the photosynthetic antennae absorbs a photon. The electronic excitation energy is then transferred through the network of light-harvesting pigments to special chlorophyll (Chl) molecules in the reaction centers, where electron transfer is initiated. Energy transfer and primary electron transfer processes take place on timescales ranging from femtoseconds to nanoseconds and can be monitored in real-time via time-resolved fluorescence spectroscopy. Studying these processes in intact leaves, despite their extreme complexity, are essential to give insights into the functional organization of the photosynthetic machinery in vivo. Here, I summarize the current knowledge on the organization of the photosynthetic machinery in plants in vivo revealed by in vivo time-resolved spectroscopy. I also cover the recent insights into understanding how plants respond to light stress.

Light-harvesting and photoprotection mechanism in cyanobacteria

Maria Agustina Dominguez-Martin[1,2,3,*], Paul V. Sauer[4,5], Markus Sutter[1,2,3], Henning Kirst[2,3], David Bina[6,7], Basil J. Greber[3,4,&], Eva Nogales[3,4,5,8], Tomáš Polívka[6] & Cheryl A. Kerfeld1,2,3,9

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Phycobilisomes (PBS) are the elaborated light-harvesting antennas in cyanobacteria. To balance the harvesting of light energy against the risks of photodamage, many cyanobacteria have evolved a photoprotective mechanism that relies on the interaction between a photoreceptor, the Orange Carotenoid Protein (OCP), and the PBS. Here we present four cryo-electron microscopy structures, with and without OCP, of the 6.2 MDa PBS from the model organism *Synechocystis* PCC 6803 at overall resolution 2.1-3.5 Å. The structures revealed the existence of three different conformational states of the antenna, two previously unknown, for the unquenched PBS. We found that two of the rods can switch conformation within the complex, suggestive of a potentially new type of regulation. We also discovered a novel linker protein, named ApcG, that binds to the membrane facing side of the PBS. In addition, the structure of the PBS-OCP complex shows four 34 kDa OCPs organized as two dimers quench the PBS. The complex also reveals for the first time, the structure of the active form of the OCP, revealing an ~60 Å displacement of its regulatory C-terminal domain. Finally, we elucidate energy transfer pathways based on structural and spectroscopic properties. These results provide detailed insights into the cyanobacterial light-harvesting and place a foundation for future bioengineering applications.

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A large-scale study of chloroplast movements and dark positioning in plants from different light environments

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Chloroplast movements are a cellular strategy for optimizing the photosynthetic efficiency of plants. In low light, chloroplasts move towards the cell wall lying perpendicular to the direction of incident light to optimize light capture. In high light, chloroplasts relocate to cell walls parallel to the direction of incident light. In the majority of land plants, they are controlled by blue light photoreceptors called phototropins. The diversity of chloroplast movements in wild species were studied by Senn, 1908[1]. In this work, we present a comprehensive analysis of chloroplast movements in wild-growing species. Over two hundred species of herbaceous angiosperm plants were collected in southern Poland, in types of vegetation characterized by different light conditions, from sun-exposed xeric grasslands, through mesophilic meadows, wetlands, ruderal communities, to the forest floor. Amplitudes and rates of chloroplast accumulation in response to 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and chloroplast avoidance induced by 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of blue light were examined through measurements of changes in leaf transmittance. Our results show that all major angiosperm families present in Central Europe contain species that exhibit distinct chloroplast dark positioning and show robust chloroplast movements. The responses are more pronounced in plants collected in the shade than in full sunlight. The magnitude of the response can be partly predicted by the indicator values for light conditions of Ellenberg's type [2, 3]. The amplitude of chloroplast movements also depends on the taxonomic affiliation of the species, with *Brassicaceae* and *Caryophyllaceae* species typically displaying substantial chloroplast relocations regardless of the type of habitat. Species with a strong accumulation response demonstrate a strong avoidance response as well, pointing out to correlation between the magnitude of both responses.

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Hydrophobic mismatch – the missing link in the regulation of photosynthetic light-harvesting?

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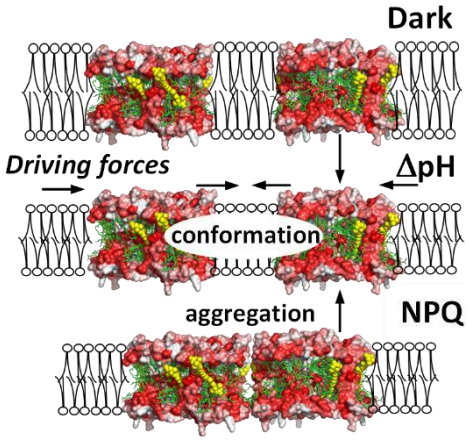
The mechanism of the prompt photoprotective process in the photosynthetic membrane of oxygenic photosynthesis, qE, is in the focus of this talk. The evidence for the central role of the major LHCII complex of photosystem II in qE will be summarized. It was shown that the minimum requirement for qE is the presence of LHCII and the proton gradient across the thylakoid membrane, whilst zeaxanthin and PsbS protein play merely a regulatory role tuning the amplitude and kinetics of the process. The novel idea that the hydrophobic mismatch between lipids and LHCII is the driver of qE has been proposed[1] (see the figure). This idea was based upon the observation that the formation of the gradient was accompanied by the thinning of the membrane that was earlier explained result of conformational changes and dehydration[2]. Experiments on using the mismatch mitigating agents cholesterol and lipids with variable acyl chain length to corroborate the proposed role of hydrophobic mismatch in qE.



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Isolation and analysis of the photoprotective locus in plants using the styrene-maleic acid copolymer

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The ability to harvest light effectively in a changing environment is a necessity to ensure efficient photosynthesis and plant growth. One mechanism to protect photosystem II and fine-tune electron transfer involves the harmless dissipation of excess absorbed photons as heat and is known as qE. This process occurs in the major light-harvesting complex of photosystem II (LHCII) and is reliant upon the allosteric modulator protein, PsbS, and the transthylakoid pH gradient. However, the exact mechanism has thus remained elusive. Here, we isolate the native photoprotective locus of PSII in different physiological states using the styrene-maleic acid copolymer. We show that the yield of LHCII aggregates that can be natively isolated correlates with the presence of PsbS and qE itself. Additionally, PsbS appears to mediate a reversible quenching of far-red emission states in the light that does not appear to affect the fluorescence lifetime of the isolated LHCII aggregates. Furthermore, the LHCII aggregates appear preferentially depleted of the thylakoid lipid DGDG. We postulate that a role of PsbS is to mediate dynamic lipid rearrangements around LHCII in order to facilitate reversible aggregation and thus a fast qE response.

Poster session Tuesday August 29

Antimicrobial PDT

P1	**Maria Bartolomeu**	A study of Wastewater Disinfection with Photodynamic Treatment and its Ecotoxicological Effects
P2	**Adelaide Almeida**	Investigation of the potential of deep eutectic solvents (DES) as alternative solvents in antimicrobial photodynamic therapy
P3	**Pietro Bertolotti**	Membrane potential modulation in bacteria via push-pull azobenzene
P4	**Nina Burduja**	Curcumin-loaded hydrogels as antimicrobial photosensitisers in the treatment of post-surgery infections
P5	**Cristina Dias**	Antimicrobial properties of marine origin materials obtained by a sustainable approach
P6	**M. Amparo F. Faustino**	Light-activated Sulfonamides for Antimicrobial Photodynamic Therapy
P7	**Andreas Fellner**	Fly into the light: Treating fruit fly Drosophila melanogaster with Photodynamic Inactivation based on Na-Mg-chlorophyllin
P8	**David T. Griffin**	The effects of delivery irradiance on the antibacterial efficacy of 222 nm Far-UVC light
P9	**Linda Jernej**	Photodynamic Inactivation in Agriculture: Combating Fungal Phytopathogens Resistant to Conventional Treatment
P10	**Beata KruszewskaNaczk**	Genes encoding proteins engaged in DNA repair are important in the response of bacteria to antimicrobial Blue Light
P11	**Michaela Kubáňová**	Photoinactivation of opportunistic pathogens in water environment with nanocomposite materials based on graphene oxide decorated with octahedral molybdenum cluster complexes
P12	**Francesca Laneri**	Photochemical evaluation of curcumin nanoformulation with BSA and its antimicrobial action in Acinetobacter baumannii
P13	**Alessia Lena**	In vitro study for evaluate the photodynamic inactivation of E. coli, L. monocytogenes and S. Enterica
P15	**Lucy Sinclair**	Bactericidal Efficacy and Cytotoxic Responses of Pseudomonas aeruginosa to Low Irradiance 405-nm Light
P17	**Annette Wimmer**	With blue light against biofilms: Berberine as natural photosensitizer for Photodynamic Inactivation of Gram+ and Gram- bacteria

A study of Wastewater Disinfection with Photodynamic Treatment and its Ecotoxicological Effects

Maria Bartolomeu[1], Thierry Gomes[1], Fabio Campos[1], Susana Loureiro[1], M. Graça P. M. S. Neves[2], M. Amparo F. Faustino[2], Ana T. P. C. Gomes[3], Adelaide Almeida[1]

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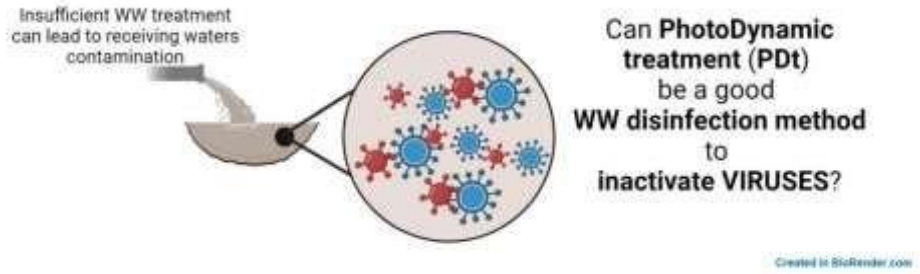
Viruses have higher mutation rates when compared with other microorganisms, particularly RNA viruses [1]. The higher mutation rate promotes the development of resistance to traditional antivirals, establishing a resistance behavior in viruses populations [1]. RNA viruses in wastewater (WW) have already been reported, leading to potential public health risks [2]. Wastewater treated with conventional antimicrobial approaches (tertiary WW treatments) like UV light, chlorine, and ozone can lead to viruses mutations and the formation of toxic by-products harmful to humans and the environment [3]. All this, highlights the inevitability to provide alternative WW disinfection techniques. Antimicrobial Photodynamic treatment (PDt), an approach based on the action of reactive oxygen species (ROS), is being considered a promising alternative to viruses inactivation without the generation of viral mutations or toxic by-products [4,5].

This study evaluated the efficiency of PDt in the inactivation of bacteriophage Phi6 (RNA-viruses model) in real WW. PDt assays were carried out in a buffer solution (PBS, as a controlled medium) and in WW (after secondary treatment) with Methylene Blue (MB) as photosensitizer (PS), and a low energy consuming light source (LED). The disinfection protocol developed with MB resulted in an efficient inactivation of the bacteriophage Phi6, both in PBS and in the real WW. Considering that treated effluents are released into the environment, the acute toxicity of PDttreated WW to the model organism *Daphnia magna* was also evaluated during a 48h exposure to the PDt-treated WW with MB.

In this communication it will be present and discuss the PDt protocol developed to photoinactivate the model RNAvirus bacteriophage Phi6 and the preliminary results of the acute toxicity of PDt-treated WW in *Daphnia magna* model.



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Investigation of the potential of deep eutectic solvents (DES) as alternative solvents in antimicrobial photodynamic therapy

Cátia Vieira[1]; Maria Bartolomeu[1]; Sónia N. Pedro[2]; Ana T. P. C. Gomes[3]; Cristina J. Dias[4]; Sara R. D. Gamelas[4]; Leandro M. O. Lourenço[4]; Carmen S. R. Freire[2]; M. Graça P. M. S. Neves[4] ,M. Amparo F. Faustino[4]; Adelaide Almeida[1]

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Antimicrobial Photodynamic Therapy (aPDT) is gaining recognition as an effective approach for the photoinactivation of a broad spectrum of microorganisms [1,2]. Due to their apolar behavior, photosensitizers (PS) often have low solubility in aqueous media requiring the use of toxic organic solvents that are incompatible with their application in the field [4]. In this study, the potential of Deep Eutectic Solvents (DES) to prepare formulations of one chlorin (bearing five cationic charges) and two porphyrins (neutral THPP, and tetra cationic Tetra-Py(+)-Me) poorly water-soluble was investigated. To this purpose, formulations of each PS were prepared in DMSO and three DES (betaine:glycerol, proline:xylitol and betaine:citric acid) and their physio-chemical properties, cytotoxicity for mammalian cells, and photodynamic action towards *Escherichia coli,* as a gramnegative bacterium model, were evaluated. The physico-chemical experiments revealed that all PS were soluble in the selected DES. However, PS stability varied highly depending on the solvent used. In dark conditions, chlorin remained stable in DES formulations while strong aggregation was observed in DMSO. In contrast, THPP showed better stability in the former solvent. Tetra-cationic porphyrin was stable in the dark regardless of the solvent used, but upon irradiation, DES formulations increased this PS photostability. Regarding the biological experiments, betaine:glycerol and proline:xylitol formulations lacked toxicity towards mammalian cells and improved Tetra-Py(+)Me photodynamic activity against *E. coli.* On the other hand, betaine:citric acid formulation, although highly toxic to Vero cells, enhanced the photodynamic action of both THPP and Tetra-Py(+)-Me. Overall, these findings indicate that DES have a high potential to prepare formulations of PS with low solubility in aqueous solutions and, in some cases, can improve their antimicrobial photodynamic activity.

Keywords: Deep Eutetic Solvents, antimicrobial photodynamic therapy (aPDT), porphyrin, chlorin, *Escherichia coli*

Acknowledgments:

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FEDER, within the PT2020 Partnership Agreement and "Compete" 2020. Thanks are also due to the Portuguese NMR and Mass Networks. C.V., S.P., C.J.D., and S.R.D.G. thanks FCT for their doctoral grants (SFRH/BD/150358/2019), (SFRH/BD/132584/2017), (SFRH/BD/150676/2020) and (SFRH/BD/143549/2019), respectively.

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Membrane potential modulation in bacteria via push-pull azobenzene

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Optomodulation uses light to artificially alter physiological or metabolic functions in organisms [1]. This approach can be a very effective and precise single-cell analytical instrument and our aim is to artificially regulate the ion exchange in bacteria [2]. In particular we photostimulate *Bacillus subtilis* after exposure NO_2–2(C_6-Pyr), a recently developed azobenzene molecule. Its exposure to visible light (470nm) leads to a trans-cis isomerisation reaction, accompanied by a strong change in the dipole moment thanks to the electron-donor acceptor moiety (Push-Pull). This in turns leads to a decrease of the membrane potential (depolarization) and a subsequent modulation of the cellular signalling.

Here, we show that a membrane-targeted azobenzene is able to modulate the membrane potential via visible light stimulation. Specifically we performed timelapse microscopy and we observed that depolarization of 5mV across the cell wall occurred within 10 seconds (figure 1) after a 10 seconds light pulse and the recovery of resting membrane potential lasted 30 seconds.

These results show our capability to influence bacteria membrane potential with optomodulation systems, which will allow us to better understand and analyze microbial physiological mechanisms.

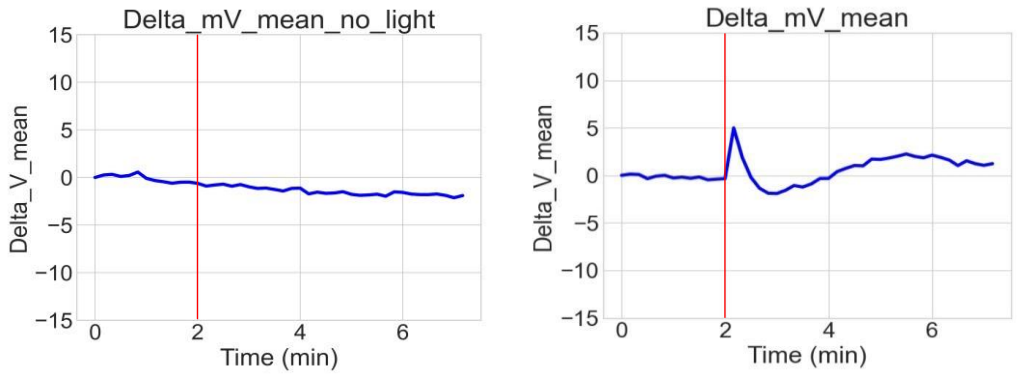

Figure 1: depolarization effect registered during the timelapse. On the right, the control, without light stimulation, on the left under a 10 seconds 470nm light pulse. The red line marks the shining of the light.

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Curcumin-loaded hydrogels as antimicrobial photosensitisers in the treatment of post-surgery infections

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Microbial infections are becoming a serious health issue due to the widespread diffusion of antibioticresistance. Multidrug-resistant bacteria can diffuse easily from person to person or from patient to patient during hospitalization and post-surgery. Therefore, therapeutic strategies and innovative antimicrobial biomaterials are being developed to effectively combat pathogens without inducing resistance and accelerate postoperative recovery[1].

As part of the ongoing research on supramolecular photosensitisers systems[2], here we report rapidly resorbable hydrogels based on DAC® (HA-PLA) loaded with curcumin I, (1*E*,6*E*)-1,7-bis(4-hydroxy-3methoxyphenyl)hepta-1,6-diene-3,5-dione (Cur) capable of increasing water solubility and bioavailability resulting in controlled release of curcumin into the infection site. Curcumin is attracting increasing interest in the treatment of bacterial infections as it has a broad spectrum of activity against both Gram-positive and Gram-negative bacteria[3]. In addition to being a photosensitizer (PS) applicable in antibacterial photodynamic therapy (aPDT). An additional hydrogel is based on DAC® loaded with Cur and Vancomycin (VAN), an antibiotic used to treat different bacterial infections including skin, bloodstream, bone and joint infections, but with limitations[4]. The combination could help overcome antimicrobial-resistance and reduce the antibiotic dosage with minimal side effects.

Our goal is to develop appropriate antibacterial surfaces based on hydrogels using biodegradable, biocompatible, and nonimmunogenic systems that combine the properties of biomaterials. Hydrogels were prepared by hydrating an organic film of Cur obtained by solvent evaporation, with an adequate co-solvent. Then, the DAC® powder was hydrated with Cur or Cur/VAN dispersion. The interaction between Cur and DAC in the DAC/Cur complex was studied using complementary techniques such as UV/Vis spectroscopy, fluorescence, Dynamic Light Scattering (DLS), ζ-potential.

The stability and erosion kinetics of the hydrogels were studied in media mimicking physiological conditions to ensure control over time and efficacy. In addition, the antimicrobial activity of hydrogels against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE) and *Pseudomonas aeruginosa* strains was evaluated using time-kill assay.

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Antimicrobial properties of marine origin materials obtained by a sustainable approach

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Natural pigments, such as chlorophylls, possess interesting properties to be used as antimicrobial agents under light conditions. Over the years, several studies have reported the ability of chlorophyll derivatives to destroy pathogenic microorganisms, like bacteria, viruses and fungi [1-2]. These pigments can be extracted from natural sources, and despite the process to obtain chlorophyll extracts can be very laborious, more sustainable, and environmentally friendly processes to obtain these pigments from natural sources have emerged [3]. Furthermore, to accomplish the Goals of 2030 Agenda for Sustainable Development, it is important to find out solutions to reduce waste and provide better and reusable materials. We found that an AmbertLite resin can be used to extract natural chlorophylls from the microalga *Isochrysis galbana* Parke 1949 and the process allows to recover carotenoids, solvents, and the resin with chlorophylls adsorbed for further reuse [4]. In this work, we will discuss the sustainable process to obtain the AmbertLite resin enriched in chlorophylls and its photodynamic action against a multidrug-resistant *Staphylococcus aureus* strain.

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Light-activated Sulfonamides for Antimicrobial Photodynamic Therapy

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Sulfonamides are the oldest class of conventional antimicrobials to combat infections. Sulpha drugs are dihydropteroate synthase competitive inhibitors, *p*-aminobenzoic acid antimetabolites and consequent inhibiting tetrahydrofolic acid synthesis which is essential to the formation of nucleic acids precursors in bacteria [1]. Light-activated porphyrin sulfonamides have emerged as a promising class of compounds with potential for antimicrobial applications [2]. These molecules can produce reactive oxygen species (ROS) upon irradiation with light, which can damage the cell wall and other cellular components of bacteria [3]. This makes them a potential alternative to traditional antibiotics, which are facing increasing resistance from microbial populations. Once the combination of different therapeutic approaches may improve the biological activity, the aim of this work was to combine a porphyrin with a sulpha moiety with an amide bond that could be cleaved by amidases naturally present in bacteria [4]. Thus, the present work describes the synthetic access and strategy for preparing porphyrin-sulfonamide bioconjugates containing enzymatically cleavable amide groups. The synthetic route and structure elucidation of the synthetic intermediates and the photosensitizers was achieved by spectroscopic methods and will also be presented as well as docking studies and photodynamic action results.

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Fly into the light: Treating fruit fly *Drosophila melanogaster* with Photodynamic Inactivation based on Na-Mg-chlorophyllin.

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Fruit flies spoil crops in agricultural settings. As conventional pesticides may generate negative offtarget effects on humans or the environment existing treatment methods need eco-friendly and safe alternatives. Photodynamic Inactivation (PDI) is based on the photosensitizer-mediated and lightinduced overproduction of reactive oxygen species in harmful or unwanted targets. We here explore the potential of PDI for combatting fruit fly pests. *Drosophila melanogaster* as well-established model organism[1] was employed in this study. We here introduce two different experimental approaches, the feed assay, where Na-Mg-Chlorophyllin (Chl, approved as food additive E140) is offered to the fruit flies as food in combination with sucrose (3%) and the spray assay, where the photosensitizer is sprayed onto the insects. We show that PDI based on Chl is able to induce mortality rates of *Drosophila melanogaster* of more than 99% with 5 mM Chl and LED illumination (8 hours incubation in the dark, radiant exposure 78.9 J/cm²) with the feed assay, or more than 95% after exposure to sunlight with the spray assay (14 hours dark incubation with 5 mM Chl and 5 hours of sunlight illumination, 532 J/cm²). For the anti-insect PDI to be effective, a drug to light interval of several hours is required, which would translate to spraying the photosensitizers in the evening for the field application. In conclusion, PDI causes high mortality rates of *Drosophila melanogaster* in both tested assays.

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The effects of delivery irradiance on the antibacterial efficacy of 222 nm Far-UVC light

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Ultraviolet-C (UVC) light, particularly around 254 nm, is often used for decontamination applications. However, recent research has indicated that wavelengths in the Far-UVC range (200-225 nm) possess similar germicidal efficacy, while posing less threat to mammalian tissue

Two primary bactericidal mechanisms have been identified for Far-UVC: disruption of critical cell functions by reducing electron transport chain activity, and production of reactive oxygen species (ROS) through photoionization, causing destructive peroxidation of cell membrane lipids. The effects of irradiance on these mechanisms, however, have so far been overlooked. This work investigated how Far-UVC irradiance and dose affect (i) bacterial inactivation; (ii) intracellular ROS production; and (iii) trans-membrane nucleic acid leakage levels.

Staphylococcus aureus and *Pseudomonas aeruginosa* were suspended in phosphate-buffered saline at 10^9 CFU.ml^{-1} and exposed to increasing doses of 222 nm light, delivered using a 'low', 'medium', or 'high' irradiance (0.3, 1.2 or 4.8 mW.cm^{-2}, respectively).

For both species at all irradiances, significant inactivation was achieved compared to non-irradiated control samples even at the minimum dose of 39 mJ.cm^{-2} ($p<0.05$), and the maximum dose of 252 mJ.cm^{-2} achieved ≥6.24-Log (>99.9999%) inactivation ($p<0.001$). Neither species demonstrated preferential susceptibility to higher or lower irradiances. Fluorescence from 6-carboxy-2', 7'dichlorodihydrofluorescein diacetate dye quantified levels of intracellular ROS. *P. aeruginosa*, at all delivery irradiances, recorded a ≥324% increase in ROS levels at the highest dose ($p=0.002$). However, a maximum increase of just 37% was seen for *S. aureus* ($p=0.009$). Trans-membrane nucleic acid leakage levels were similar for both species, when measured spectrophotometrically, with no observable dependence on irradiance. For *P. aeruginosa*, this increase peaked at 54.8% ($p=0.027$); for *S. aureus* at
39.7% ($p=0.019$).

Overall, results demonstrate that levels of bacterial inactivation, ROS production and cell membrane damage are independent of Far-UVC irradiance and a function of dose alone; Far-UVC induces significantly higher levels of ROS in *P. aeruginosa* than *S. aureus*; and, at given Far-UVC doses, similar increases in cell membrane damage occur for both species.

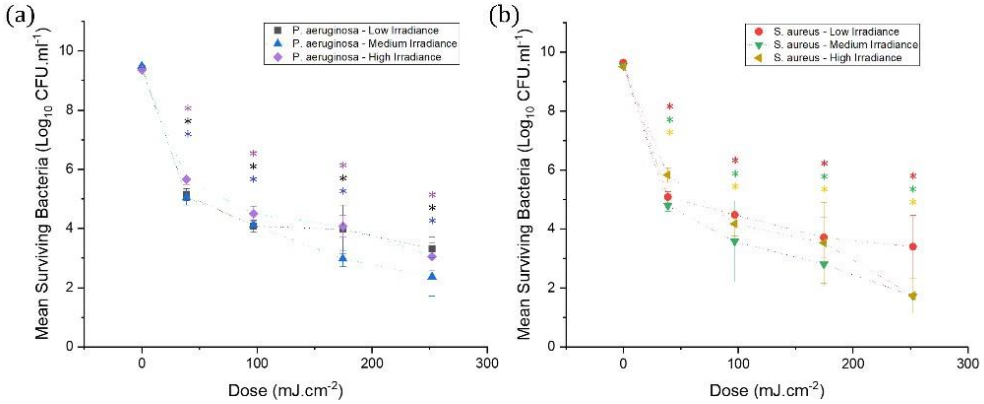

*Figure 1: 222 nm inactivation of (a) P. aeruginosa and (b) S. aureus using 'Low', 'Medium' and 'High' irradiances (0.3, 1.2 or 4.8 mW.cm-2, respectively). *denote significant ($p<0.05$) inactivation compared with non-irradiated control value (paired t-test, Minitab v18).*

Photodynamic Inactivation in Agriculture: Combating Fungal Phytopathogens Resistant to Conventional Treatment

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Worldwide 10-15% of annual crops are lost due to plant diseases of which 70-80% are caused by plant pathogenic fungi. Moulds and blotch diseases caused by *Botrytis cinerea* and *Zymoseptoria tritici* respectively, affect yields and food quality. As a countermeasure, fungicides are applied regularly in agriculture, leading to emerging resistances against classical treatment methods over the past decades[1,2]. Therefore, new treatment strategies against plant pathogenic fungi must be found. Photodynamic Inactivation (PDI) was recently introduced as a method to effectively kill plant bacteria, even if resistant to antibiotics[3]. In this study, the photokilling efficiency of PDI based on the natural photosensitizer sodium-magnesium-chlorophyllin (EU food additive E140[4]) combined with different surfactant molecules and a chelator[5], is tested against fungal plant pathogens susceptible and resistant to conventional treatment. The photoactive compound was used in concentrations between 0.015 and 0.0.70% and illumination of different strains of *B. cinerea* and *Z. tritici* was done using an LED array with 395 nm. Depending on the concentration of chlorophyllin, samples of mycelial spheres could be killed effectively. A total kill of mycelial spheres of the susceptible wild type *B. cinerea* was achieved using chlorophyllin concentrations of 0.0325 and 0.015%. Strains resistant to different classical fungicides were even more susceptible to PDI. These findings strengthen the idea of using PDI with natural PS in agriculture to fight resistant fungal plant pathogens.

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Genes encoding proteins engaged in DNA repair are important in the response of bacteria to antimicrobial Blue Light

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Antimicrobial Blue Light (aBL) is a promising strategy to fight microbial infections, especially those caused by antibiotic-resistant bacteria. However, the mechanism underlying aBL bactericidal action is still not fully understood, which can be one of the causes of not widely employing this approach. One of the hypothesis emphasizes the role of reactive oxygen species generated during blue light irradiation which cause damage to various cellular structures such as DNA, proteins, and membranes (1, 2). Therefore, different repair systems can be important in the cellular response to aBL. In this study, we focused on the role of different DNA repair mechanisms involved in protecting bacterial cells against aBL.

We have investigated 6 single-gene Escherichia coli mutants from Keio Knockout Collection (3): *umuD* (DNA polymerase V protein), *rbfA* (30S ribosome binding factor), *oxyR* (oxidative stress regulator), *purA* (adenylosuccinate synthetase), *fimB* (type 1 fimbriae regulatory protein FimB), and *deoB* (phosphopentomutase). All of the selected mutants revealed an especially sensitive phenotype to aBL in comparison to the wild-type strain. Next, ASKA plasmids (4) with genes of interest were isolated and complementation of deleted genes was performed. Interestingly, complementation restored the wildtype phenotype for *umuD, fimb, deoB* mutants. In the case of *rbfA, purA, oxyR* complemented mutants, they were even less susceptible to aBL than the wild type. This may confirm the important role of the deleted genes in the mechanism of bacterial cell protection against the bactericidal effect of aBL. The current study may contribute to a better understanding of the mechanism of aBL.

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Photoinactivation of opportunistic pathogens in water environment with nanocomposite materials based on graphene oxide decorated with octahedral molybdenum cluster complexes

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New antibiotic approaches are urgently needed to combat the rising threat of antimicrobial resistance. Traditional antibiotics are becoming less effective due to the emergence of resistant strains, necessitating the exploration of innovative strategies. The photoinactivation of pathogenic bacteria is a promising method based on materials that create large number of reactive oxidative species upon visible-light irradiation. Due to its mechanism of action, PDT has shown a low propensity to induce resistance in microbial population as it targets several critical components of bacterial cells.

Graphene oxide (GO) in the form of nanosheets has emerged as a promising nanomaterial for photodynamic therapy thanks to its unique properties, such as large specific surface area providing space for the attachment of photosensitizers, electrical and mechanical properties. The inclusion of oxygen within carbon sheets modulates electrical properties and hydrophilicity, making GO dispersible in aqueous media acting as supporting phase for further colloids such as octahedral molybdenum clusters to prepare nanocomposite materials.

Octahedral molybdenum cluster complexes (Mo_6) are efficient singlet oxygen photosensitizers with previously proven antimicrobial effect upon blue light activation. Furthermore, molybdenum clusters can be functionalized with different ligands resulting in modified properties and targeting towards bacterial or tissue cells.

This work describes the antibacterial effect of nanocomposite materials designed by association of the Mo_6 cluster compound $[Mo_6I_8(OCOC_4H_8PPh_3)_6]Br_4$ with GO sheets in the form of colloidal system as well as deposited layer on glass surface targeting opportunistic pathogens. We proved a significant phototoxicity against planktonic forms of gram-positive opportunistic pathogens such as *Staphylococcus aureus*. Moreover, in the form of active layers these materials act as an efficient inhibitor of biofilm formation, resulting in potential application for microbial inactivation in water treatment.

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Photochemical evaluation of curcumin nanoformulation with BSA and its antimicrobial action in *Acinetobacter baumannii*

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Bacterial infection is a common occurrence in cutaneous leishmaniasis lesions, and bacterial resistance becomes the biggest threat to human health in the 21st century, with an estimated 10 million deaths from resistance by 2050. Infections with resistant bacteria increase a patient's hospital stay, increase costs and increase the risk of death. There are also projections of increased resistance to second- and third-line antibiotics between 2005 and 2030 (UNICGAR, 2019). The search for alternative therapies is essential to avoid the emergence of bacterial resistance, and photodynamic therapy (PDT) is an alternative. Curcumin is a pigment extracted from turmeric (Curcuma longa) and widely used in cooking as a preservative and dye, extracted from the rhizome of Curcuma longa. Its medical applications have been studied for more than two centuries, including anticancer properties, microbiological control and in the treatment of Alzheimer's, due to its antioxidant, anti-inflammatory and neuroprotective properties (LEITE et al., 2014; PRIYADARSINI, 2014).

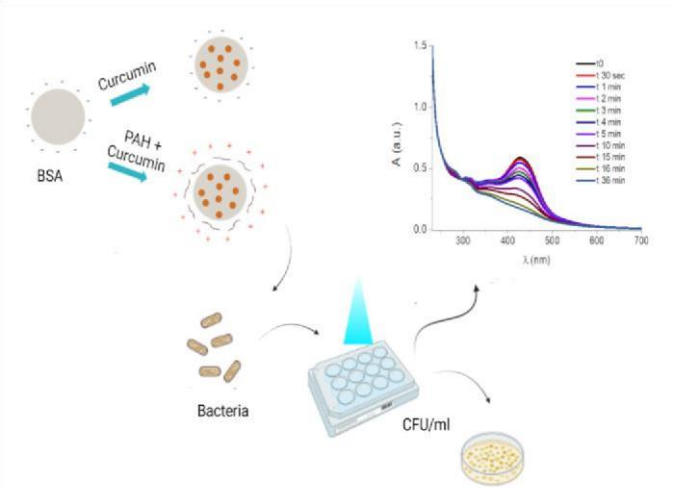

Figure 1: Synthesis process of curcumin compounds with BSA with positive and negative charge and scheme of biological and photochemical tests.

Assays of bacterial metabolism by conversion of resazurin showed that at higher concentrations, such as 1.0 and 0.7 µM, it was toxic, even in the dark. However, at lower concentrations, a photodynamic effect is observed (1D), without toxicity in the dark. Formulations with curcumin show promising results for microbial control.

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In vitro study for evaluate the photodynamic inactivation of E. coli, L. monocytogenes and S. Enterica

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Recently Photodynamic Inactivation (PDI) has been studied as a non-thermic method for sanitizing food environments. PDI is based on the action of Blue Light (BL, 400-480 nm), oxygen and an endogenous or exogenous *Photosensitizer* (PS) (Amin et al., 2016; Endarko et al., 2012). The PDI efficiency against microbes depends on the LB dose (D), wavelength, and microbial species. PDI efficacy against several microbial pathogens has been demonstrated (Leanse et al., 2018).

This study aims to investigate the inactivation capacity of blue LED lights at 405 nm, 420 nm and 450 nm against *E. coli*, *L. monocytogenes* and *S.* Enterica at different light doses. The first part consisted on a qualitative screening to evaluate the inactivation capacity of the blue LED lamps (BL) testing different doses. In the second part, a quantitative assessment of the direct decontamination capacity was done on solid and liquid media testing different conditions such as doses, treatment time, and lamp's voltage.

The qualitative screening underlined that for light doses < 700 J/cm^2, the inactivation effect was different based on the wavelength, the light dose, and the microbes. The quantitative assessment on solid substrate showed that at all the wavelengths, with a LB dose > 400 J/cm^2 all microbes were inactivated. Decreasing the LB dose, the most effective wavelength for all the microbes was 405 nm, which inactivated from -1.13 to -7.34 log depending on the microbial species. However, the 450 nm lamp showed the lowest microbial inactivation for all the bacteria.

The assessment in liquid substrate demonstrated a microbial inactivation efficacy. After 120 min, 405 nm wavelength exerts the highest microbial inactivation: *E. coli* decreased > 4 log, *L. monocytogenes* > 3 log, and *S.* Enterica > 2 log.

On solid and liquid substrates, the inactivation was dependent on the behaviour of the different microbial species.

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Bactericidal Efficacy and Cytotoxic Responses of *Pseudomonas aeruginosa* to Low Irradiance 405-nm Light

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Violet-blue 405-nm light provides a safe approach to environmental decontamination when employed using low irradiance levels. Bacterial inactivation by 405-nm light occurs due to the photoexcitation of intracellular porphyrin molecules which incites the production of reactive oxygen species that impart oxidative damage. The precise cellular targets, particularly for low irradiance exposures, are not yet fully understood. This study investigates the bactericidal efficacy of 405-nm light and the equivalent cytotoxic responses to exposure at varying irradiances. *Pseudomonas aeruginosa* was exposed to increasing doses of 405-nm light at three irradiances (5, 50 and 150 mW cm^{-2}). Inactivation kinetics at equivalent doses were established and cytotoxic responses were assessed by spectrophotometric quantification of nucleic acid leakage to indicate membrane damage and spectrofluorimetric quantification of green fluorescence from carboxy-H_2CDFDA to indicate intracellular oxidative stress. Susceptibility was significantly enhanced when exposed at lower irradiance: 108 J cm^{-2} achieved 99.9% reductions using 5 mW cm^{-2} compared to 80.2% using 150 mW cm^{-2} ($P \leq 0.001$). An upward trend in nucleic acid release and oxidative stress levels was demonstrated in all cases as applied dose was increased, however, 5 mW cm^{-2} exposures demonstrated lower nucleic acid leakage ($P \leq 0.05$) and higher oxidative stress levels ($P \geq 0.05$) compared to higher irradiances. This study demonstrates the enhanced bactericidal efficacy of low irradiance 405-nm light and identifies its association with lower nucleic acid leakage and higher cellular oxidative stress levels compared to higher irradiances, suggesting that the cell membrane is unlikely the primary target of low irradiance 405-nm light inactivation.

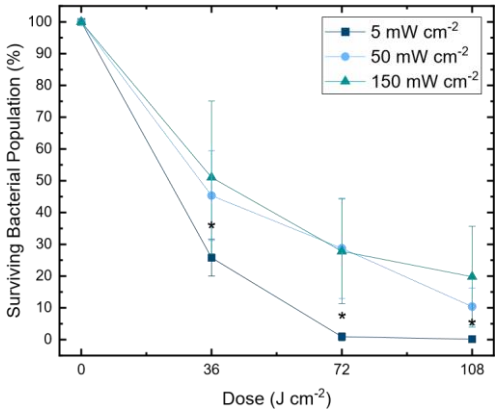

Fig 1. Inactivation kinetics of *P. aeruginosa* upon exposure to increasing doses of 405-nm light at irradiances of 5, 50 and 150 mW cm^{-2} ($n \geq 3 \pm$ SD). * indicates data points at which the bacterial reduction achieved by exposure to 5 mW cm^{-2} is significantly greater than that achieved at all other irradiance applications ($P \leq 0.05$).

With blue light against biofilms: Berberine as natural photosensitizer for Photodynamic Inactivation of Gram+ and Gram- bacteria

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Evolving antibiotic resistance in bacteria is an ongoing serious health problem and the search for alternative treatment strategies captures the attention of scientists around the world. Berberine (Ber), extracted from Barberry (*Berberis vulgaris*) and many other plants, is a natural compound famously known in Traditional Chinese Medicine for various applications. As medicinal herb, it possesses antioxidant, anti-inflammatory, anti-diabetic, anti-obesity, and anti-cancer as well as antimicrobial effects. Photodynamic Inactivation (PDI) represents an innovative method for killing microorganisms and has been proven effective against a wide range of pathogens[1]. The aim of this study is to investigate whether photoactivity of Ber is applicable for PDI treatment against relevant human pathogens such as *Staphylococcus capitis*, *Staphylococcus aureus*, and *Escherichia coli* – as planktonic cells as well as in biofilms. The number of planktonic *S. capitis* cells will be reduced by 7 log steps using 100 µM Ber (5 min incubation, illumination using 435 nm LED array resulting in a radiant exposure of 25 J/cm²). For an antibacterial effect of 4 log steps, static *S. capitis* biofilm formations require 1 mM Ber as well as 100 min incubation and a radiant exposure of 100 J/cm². *S. aureus* can be photo-killed by 5 log steps using 100 µM Ber and 25 J/cm². Prolonging the Ber incubation from 5 to 30 min leads to a total kill of *S. aureus* planktonic cells. Static biofilms of *S. aureus* can be effectively killed (7 log steps) by using 100 µM Ber photoactivated with 25 J/cm². Photo-treatment with Ber of Gram(-) *E. coli* is successful only in presence of a cell wall permeabilizing agent. An antibacterial effect of 3 log steps is detectable after photo-activation of 10 µM Ber (5 min incubation) with a radiant exposure of 25 J/cm². However, Ber failed to perform successful PDI on *E. coli* static biofilms. This paper highlights the photo-efficacy of Ber on different bacterial human pathogens. This paper may be helpful in advancing the disease management of drug-resistant bacteria using Ber in the scope of photodynamic treatment[2].

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2. to be published

Innovations in phototherapy and PDT

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Enhanced cellular delivery of rose bengal and nile red by reduction sensitive molecular nanogels

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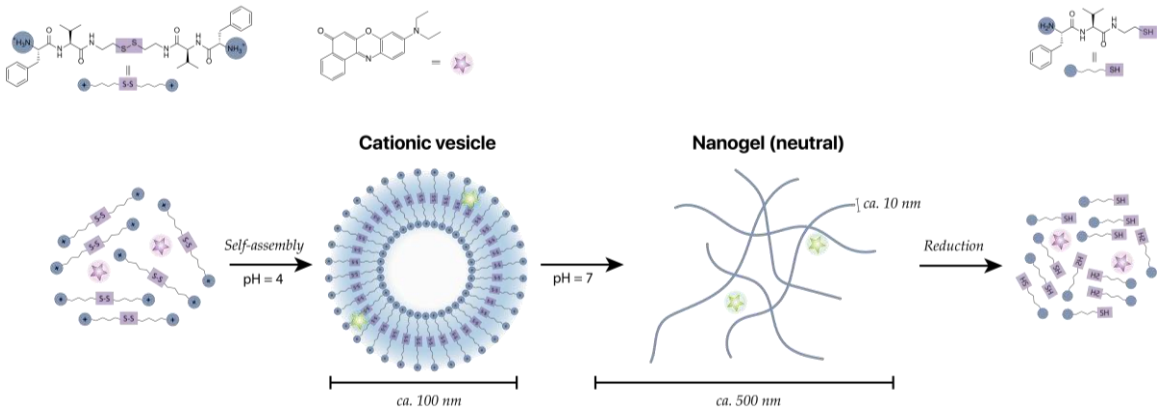
Organic nanoparticles have gained attention as vehicles for delivering substances in biomedical applications. Among these nanoparticles, nanogels stand out as a promising group of organic nanostructures. They are promising carriers due to their flexibility and compatibility with biological systems.[1]

Following our previous work on Nanogels[2], here, we report the aggregation of some peptidederived bolaamphiphiles containing a disulfide moiety. We have proved that these nanogels can be formed through a pH-induced transition from vesicles. Furthermore, due to the reduction-responsive nature of these nanogels, it enables the release of encapsulated cargo upon exposure to a reducing agent. The potential of these nanogels as carriers for Nile Red and Rose Bengal dyes in cell cultures has been evaluated, revealing enhanced cellular uptake and improved photodynamic activity, particularly in the case of Rose Bengal, compared to free solutions.



Schematic representation of vesicle to nanogel transition and reduction triggered disassembly

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Antibacterial photodynamic capability of conjugated polymer nanoparticles of CN-PPV

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The photoantimicrobial properties of conjugated polymer nanoparticles (CPNs) composed of poly(2.5di(hexyloxy)cyanoterephthalylidene) (CN-PPV) were investigated. The CPNs were prepared via nanoprecipitation using varying ratios of organic and aqueous phases and different concentrations of polysorbate 20 (tween® 20) in the aqueous phase [1]. By using a 2:10 tetrahydrofuran (THF):H_2O ratio and increasing the concentration of tween 20, smaller CPNs were obtained, and the presence of the surfactant led to higher CN-PPV yields and slightly electronegative CPNs. Under illumination at 450 nm, the CPNs generated reactive oxygen species, with the 2:10 CPNs being more efficient and photostable than the 1:10 CPNs. All CPNs induced cell death of *S. aureus* and *E. coli* in the presence of light, with the CPNs produced without and with tween 20 at the 2:10 THF:H_2O ratio presenting the best photoantimicrobial activity against both Gram-positive and Gram-negative bacteria.

Acknowledgments: CNPq, CAPES, FUNDECT, Sisfóton-MCTI, and UFMS.

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Increasing cancer permeability by photodynamic priming: From microenvironment to mechanotransduction signaling

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Therapeutic agents for cancer treatment often find their efficacy hindered by the dense tumor stroma, which prevents the appropriate diffusion of various therapeutics molecules including nanotherapeutics beyond tumor periphery. Photodynamic priming (PDP) has emerged as a novel approach that uses low dose photodynamic therapy (PDP) to induced enhanced permeability in cancer tissues[1-2], this effect has already demonstrated to improve chemotherapy efficacies[3-5]. Nevertheless, the exact mechanisms by which this technique works are not fully investigated. We conducted exhaustive bibliographic research into the effects of PDT on the extracellular matrix, mechanoreceptors (e.g., integrins), and mechanotransduction pathways (e.g., FAK/Rho/ROCK). Upon analyzing 318 research papers, we found that PDT/PDP can cause extensive oxidative damage within the cancer microenvironment. PDT has the capacity to denature extracellular matrix proteins, reduce integrin and CD44 mechanoreceptor levels, and interfere with various downstream signaling pathways. Despite several identified knowledge gaps, the findings convincingly show that PDT degrades the extracellular matrix reducing the ability of cancer cells to translate extracellular signaling into cell behavior, preventing epithelial-to-mesenchymal transition. PDP holds strong potential to increase cancer permeability and increase the efficacy of adjuvant chemotherapeutics. A more comprehensive insight into the molecular mechanisms of PDP was obtained, which can aid in further developing this approach in a safe and effective manner.

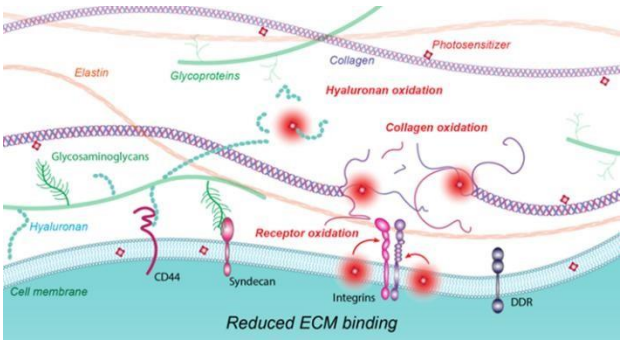

***Figure 1**. The impact of PDT on the extracellular matrix components.*

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Pyrylium salts as fluorescent probes for mitochondrial imaging

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Due to their synthetic, photophysical, and analytical aspects, pyrylium salts and their analogues, pyridinium and thiapyrylium salts (Fig.1), are heterocyclic molecules of great interest. These are structural units found in various natural products and biologically active compounds [1]. The various possible structural modifications allow for the derivation of compounds with different photochemical activities. Due to their diverse chemical properties, particularly their characteristic optical properties, these compounds are promising as markers for cellular organelles such as mitochondria [2][3][4].

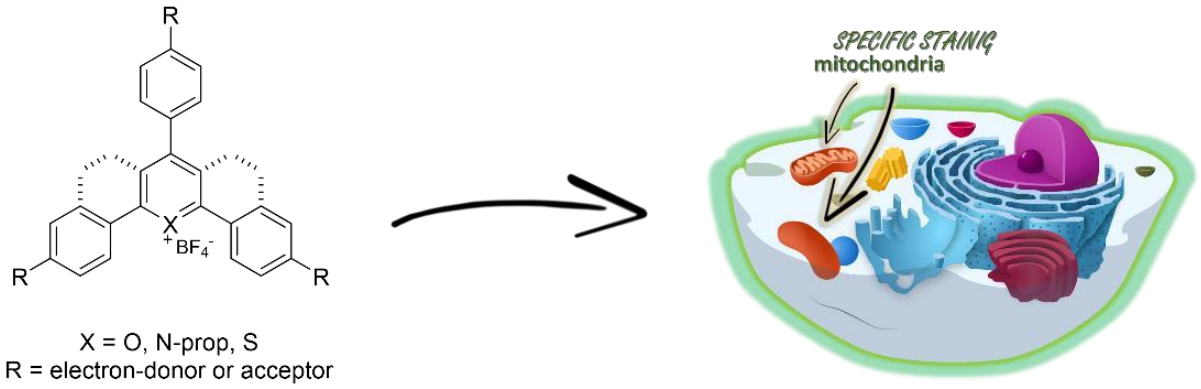

Fig. 1. Illustration of the use of fluorescent probes for mitochondrial labeling

In this way, fluorescent cellular probes from pyrylium salts and their analogues were synthesized and characterized through absorption and emission studies, Nuclear Magnetic Resonance spectroscopy, and cellular colocalization assays using Confocal Laser Scanning Microscopy and Flow Cytometry with live cells. The results demonstrate the applicability of these probes as efficient markers of cellular organelles, making them promising tools for photobiology and photochemistry research.

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In vitro phototherapeutic effects of four new *N*-propylbenzene squaraine dyes against prostate cancer cells

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Squaraine dyes are a class of compounds that exhibit some characteristics inherent to those of an "ideal photosensitizer", such as absorption at near-infrared-close wavelengths and the ability to produce reactive oxygen species. The introduction of amine groups into the four-membered central ring, although known as increasing the phototoxicity of squaraines, can improve squaraines' water solubility and induce bathochromic shifts compared to their unsubstituted zwitterionic derivatives, interesting effects in biological contexts. From a biological point of view, due to their cationic nature at physiological pH, aminosquaraine dyes can also have a higher cellular uptake and mitochondrial preferential accumulation. In this work, three new aminosquaraine dyes (Figure 1, (**CC-4** to **CC-6**) were synthesized, fully characterized, and evaluated concerning their potential application as cancer PDT photosensitizers. The effects of the new dyes were assessed against the PC-3 prostate cancer cell line, and its cytotoxicity against normal human dermal fibroblasts (NHDF) was evaluated to determine their possible tumor selective effects, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Light-emitting diode systems centered at wavelengths close to the dyes' maximum absorption wavelengths were used for irradiation.

Finally, it was possible to conclude about the dye that provides the best *in vitro* phototherapeutic response and which structural modification offers the best potential as PDT attractiveness.

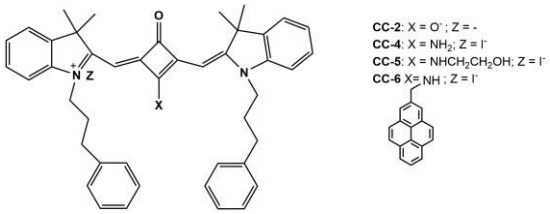

Figure 1. *N*-propylbenzene benz[*e*]indole-based squaraine dyes.

Keywords: squaraine dyes, photosensitizers, prostate cancer, photodynamic therapy

Acknowledgments: C. Costa would like to thank the Calouste Gulbenkian Foundation, particularly the "Novos Talentos" program, for funding her participation in this Congress. E. Lima is grateful to the Portuguese Foundation for Science and Technology for his PhD grant SFRH/BD/147645/2019.

Designing *in vitro* aPDT experiments from A to Z by optical methods only

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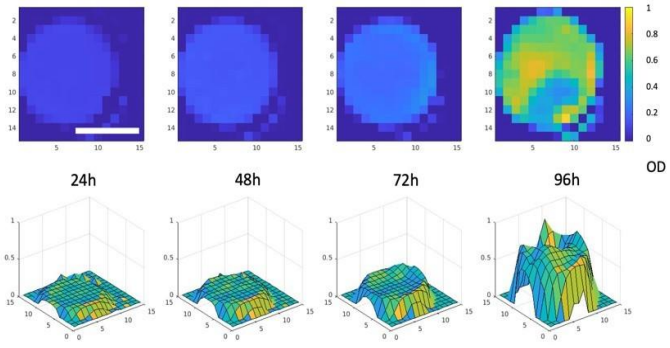
In antibacterial PDT (aPDT) *in vitro* experiments are generally divided into: (i) sample preparation; (ii) irradiation with λ_{EX}; (iii) measurements of the photokilling efficacy. In each stage variable conditions can be considered, such as: biofilm age, medium, growth surface and temperature in sample preparation; light dose, photosensitizer type and concentration in irradiation. Step (iii) is generally performed by Colony Forming Unit (CFU) counting. If irradiation is inherently associated to the use of optical methods, the other steps do not generally imply them at all, if not for the quantification of the initial bacterial optical density (OD). Being aPDT based on light-biological matter interaction, optical methods could be increasingly used in: (1) sample preparation, to analyze the variability in biofilm morphology and light absorption by inter- and intra-biofilm OD(λ_{EX}) measurements; (2) efficacy assessment with live/dead molecular probes.

Our aim is to propose and discuss an approach to aPDT with *in vitro* biofilm samples, based on a commercial microplate reader (Infinite M200PRO - Tecan) to assess the biofilm conditions and their reproducibility, and estimating the photokilling efficacy. Preliminary results in terms of biofilm morphology and optical density profile at two representative wavelengths λ = 405 and 600nm were obtained with *E. coli* biofilm (K12 strain), grown in Luria-Bertani broth, on both agar and plastic surfaces for 24h, 48h, 72h and 96h at 37 °C. At each time, CFU counting was performed and the biofilm OD_{405} and OD_{600} were measured in Phosphate buffered saline with a ~ 400µm resolution. The OD (x, y) profile (Figure 1) and CFU value were associated to the biofilm growth conditions. Further steps will concern biofilm sensitization, irradiation, and measurement of the photokilling efficacy. The proposed experimental approach aims at a better standardization of aPDT experiments, evidencing the role of different initial conditions on the photokilling outcome and proposing a possible alternative to time-consuming methods such as CFU counting.



Synthesis of hemiindigoids as photoswitchable entities: a spatiotemporal handling of enzyme activity using light

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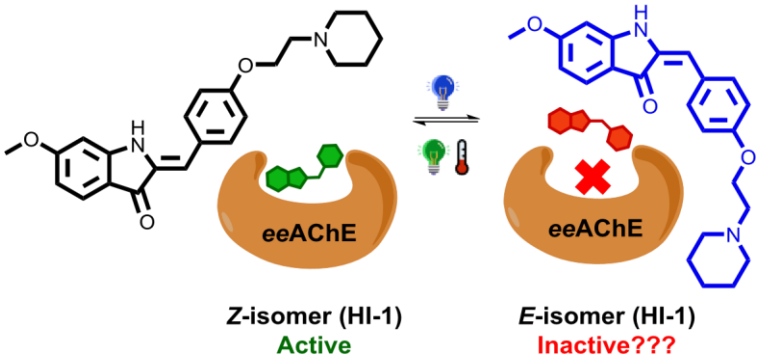
Keywords: Molecular photoswitches, hemiindigoids, acetylcholinesterase, photopharmacology, light.

Hemiindigoids comprise a range of molecular scaffolds mainly represented by aurones, indanones, hemiindigos (HI) and hemithioindigos (HTI). These scaffolds have been associated with many biological activities, such as enzyme inhibition in several models, and studies consecrated to the elucidation of their medicinal potential revealed promising results over the past years. Interestingly, these derivatives possess intrinsic optical properties and in particular, they can have a photochromic behavior, *i.e.* reversible isomerization reactions can be triggerred by light illuminations. In particular, when irradiated with light of appropriate wavelength, hemiindigoids can change their molecular geometry from the thermodynamically stable *Z*-isomer to the metastable *E*-isomer[1]. Expecting that enzymes will be able to discriminate between the two isomers, this work aims to develop hemiindigoids for photopharmacology, a modern branch of pharmacology where light is used as a non-invasive stimulus to control biological activity and, thus, avoid off-target effects. In this context, we synthesized and tested a series of hemiindigos as inhibitors for acetylcholinesterase (AChE) as a model enzyme. A proof-of-concept was obtained in the frame of AChE inhibition, where the results of biological assays performed in the dark (pure *Z*-isomer) and upon blue light irradiation (450 nm) showed an IC_{50} light/dark ratio of 1.8 for HI-1.



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Synthesis and connection of two chromophores: a promising Glyco-OPE-Porphirin and its aggregation properties.

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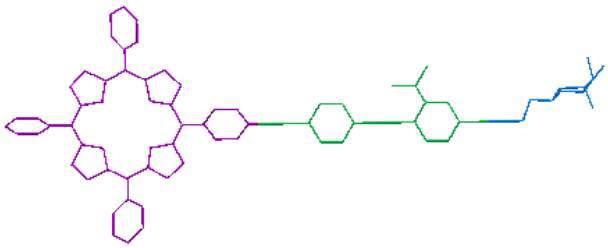
Porphyrins are heteroaromatic heterocyclic chromophores consisting of four pyrrole subunits linked by methin bridges at their alpha-carbon. These compounds are involved in oxygen transport in animals' blood and in chlorophyll photosynthesis in plants. Porphyrins have been deeply studied as photosensitizers (PS) in photodynamic therapy (PDT) and biosensors in several disease's diagnosis. [1,2] Oligo phenylene ethynylenes (OPEs) are luminescent compounds consisting of aromatic rings alternating to triple bonds; the peculiar photophysical features of these systems, allowed their application in a many fields. Recently, our group has studied and reported the synthesis of Glyco ammino-OPEs and their use as PS in PDT.[3]

This communication is about the design, synthesis and characterization of a new glucose-terminated OPE-Porphyrin dichromophoric system, with an in-depth analysis of its aggregation properties. OPE moiety is not only a linear spacer between the porphyrin and the sugar termination, since it can influence photophysical properties and aggregation behavior of the system. Both probes tend to establish non-covalent interactions, allowing the generation of new supramolecular aggregates. The glucose termination guarantees amphiphilic characteristics to the system, that can be promising in drug delivery, cellular targeting, PDT and bioimaging.



3D representation of a glucose-terminated OPE-Porphyrin.

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2 A. Romeo, M. A. Castriciano, L. Monsù Scolaro, Int. J. Mol. Sci. 2021, 22.
3 C. M. A. Gangemi, A. Barattucci, P. M. Bonaccorsi, Molecules 2021, 26, 3088 – 3121

Evaluation of the photodynamic effect of red wine-inspired pyranoflavylium cations (HPF) sensitizers.

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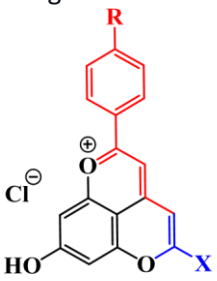
Introduction: Cancer is the disease that causes the second highest number of deaths worldwide, being only inferior to cardiovascular diseases[1]. Cancer is remarkably deadly and the treatments routinely applied suffer from high rate of undesirable effects[1]. In the pursuit of newer and less invasive treatments, Photodynamic therapy (**PDT**) has emerged as an attractive option. Therefore, designing new photosensitizers (**PS)** for **PDT** is crucial. In this context, nature can be an attractive source of inspiration. One example is the use of Pyranoflavylium cations (**HPF**), synthetic analogs of Pyranoanthocyanins formed by the reaction of red wine anthocyanins with yeast secondary metabolites. The photophysics of **HPF** such as the singlet oxygen quantum yield and the photoredox proprieties pointed to the possibility of their use as a new class of PDT sensitizers [2]. **Objectives:** Evaluation of the phototoxicity and cytotoxicity of six different substituted Pyranoflavylium cations (Figure 1: **1**: p-OCH3; **2**: p-CH3; **3**: Trimethoxy; **4**: Naphthyl; **5**: p-F; **6**: p-Br and p-I). Optimization of experimental conditions and phototoxicity effects. Study of intracellular localization, PS uptake and damage caused. **Methodology:** For phototoxicity and dark toxicity, HeLa cells were seeded in two 96 well plates, incubated for 24 hours and treated with (**1-6**) for 1-2 h. The plates were irradiated using a Biolambda LED at 450 nm for 30 minutes. The dark control was kept in the dark for 30 minutes. The cellular viability was determined 24 or 48 h later using the MTT viability test. For the **colocalization assay**, HeLa cells were seeded in 24 well plates, incubated for 24 hours, and treated with (**1-6**) for 1 hour. The cells were then treated with Lyso TrackerTM Deep Red or Mito TrackerTM Deep Red and images acquired using a Leica epifluorescence microscope. The PS uptake was evaluated as described in MARTINS [2]. **Results:** The **HPF** treatment showed a mild to strong phototoxicity, with **LD50s** of 0.85, 1.3, 1.2; 10.0, 16.6 and 26.4 µM for **1** to **6** respectively, and no appreciable Dark toxicity. Is worth noticing that the **LD50s** values increase with the increment of Hammett sigma constants. Increasing the incubation and evaluation time decreased the **LD50** values. The analysis of the fluorescence microscopic images showed a tendency of **HPF** to localize in mitochondria and lysosomes.



1: X= p-OCH_3phenyl; **R**=H
2:X= p-CH_3-phenyl; **R**=H
3: X= Trimethoxy-phenyl ; **R**=H
4: X= Naphthyl; **R**=H
5:**X**= p-F-phenyl; **R**=H
6: X= p-Br-phenyl; **R**=I

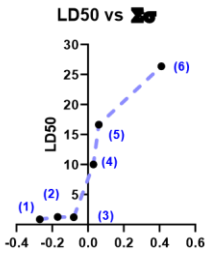


Figure1. Structures of **HPFs 1-6** and **LD50** values for PDT cell death of HeLa cells plotted against the Hammett sigma constants of the substituents R and X.

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5-ALA Mediated PDT/Radiotherapy Combination for Breast Cancer Using Gold Chalcogenides

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Radiotherapy (RT) is a critical treatment method used in medical facilities to effectively manage and eliminate inoperable tumor areas [1]. To eliminate cancer cells, a high-energy dose of ionizing radiation is necessary, but this can cause damage to healthy surrounding tissues [2]. Additionally, if the radiation dose is reduced or the number of radiation sessions is increased, it can lead to the development of radiation resistance in cancer cells, reducing the effectiveness of RT and potentially causing treatment failure [3, 4]. The combination of the treatment with photodynamic therapy (PDT) with the delivery of a photosensitizer will overcome the resistance to RT and increase therapeutic efficiency with lower doses of radiation. We developed highly stable gold chalcogenides (GCs) and demonstrated their potential as radiosensitizers. These nanoparticles have surface plasmon peaks in visible and nearinfrared spectral regions, allowing wavelength-selective light irradiation. In this study, branched polyethyleneimine-coated GCs were synthesized and loaded with 5-aminolevulinic acid (5-ALA), which is a precursor of PDT agent protoporphyrin IX (PPIX), to achieve enhanced combination therapy of PDTRT. *In vitro* cytotoxicity of bPEI-GCs, ALA-bPEI- GCs, and free ALA was evaluated for the MDA-MB-231 cell line, and the most suitable conditions were selected. MDA-MB-231 cells showed the non-toxic nature of the particles. PDT-RT combination therapy was applied via irradiation of the cells with 640 nm with light emitting diode at 10 mW cm^{-1} power, for 1 minute and then exposed to 2 Gy radiation. Cell death was evaluated with a standard MTT assay. Further, cell death mechanisms were investigated in terms of ROS generation, apoptosis/necrosis, and live/dead imaging. This study was focused on lowering the radiation dose and enhancing the phototherapy effect on breast cancer.

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A Type-I Photosensitiser for Hypoxic Photodynamic Therapy

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Typical photodynamic therapy (PDT) is known to be highly dependent on oxygen concentration, hampering its effectiveness as a promising approach to cancer treatment[1], as solid tumors are inherently hypoxic. Developing oxygen-independent photosensitisers (PSs) for producing reactive oxygen species (ROS) in the absence of oxygen, is expected to fundamentally solve the problem of the oxygen dependence of PDT. In type-I PDT, the excited PS transfers electrons to the surrounding molecules producing radicals. In contrast, type-II PDT involves energy transfer from the excited triplet-state PSs to the ground-state molecular oxygen, resulting in the generation of singlet oxygen ($^1O_2$). Type-II PDT is more dependent on oxygen concentration and therefore shows limited therapeutic efficacy in hypoxic microenvironments. Thus, type-I PDT has been shown to have great potential, as it requires less oxygen input. We have identified a molecular Type-I photosensitizer that produces hydroxyl radicals ($HO^{\bullet}$) under a hypoxic (2% O_2) microenvironment. It is noteworthy that $HO^{\bullet}$ is the most reactive ROS in biosystems, thus its production should improve the outcome of PDT. We have tested its potential *in vitro* in MCF-7 cells by varying the oxygen concentration during the photodynamic treatment. Indeed, the studied photodynamic agent is more phototoxic under severe hypoxic conditions (CC_{50} of 6.3 and 17.84 □M at 2% and 21% O_2, respectively), in agreement with the photochemical studies.

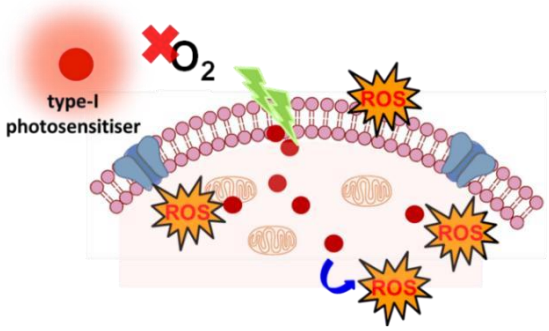

Figure 1. Graphical abstract of the present work

Acknowledgment: This research was funded by the Agencia Estatal de Investigación and FEDER (ref. PID2020-115801RB-C22)

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Indazole from medicinal chemistry to selective photosensitizers

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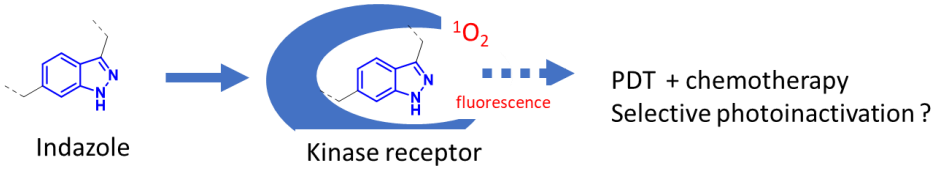
Generally, the photosensitizing nature of medicinal compounds is perceived as a drawback. In particular, kinase inhibitors often consist of aromatic rings exhibiting a non-negligible absorption in the UV. However, this defect could be turned to advantage by allowing the development of molecules generating ROS under irradiation, also featuring a good affinity and selectivity toward a specific receptor. This should improve the targeting of PDT, permitting to inactivate a receptor or to combine it with chemotherapy.

For this purpose, we are currently working with indazole moiety which is one of the most important heterocyclic systems in drug development. The presence of two nitrogen atoms favors interactions with biomolecules and it is already involved in many commercially available drugs. However, chromophores based on this structure are still scarce, therefore, in order to explore its potentiality, we synthesized a family of push-pull molecules using indazole as electron donating groups. This allowed us to obtain effective photosensitizers with high singlet oxygen quantum yields.



Combining PDT and chemotherapy using a single compound.

Antibacterial photodynamic and sonodynamic therapy – in vitro study

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Resistance to antibiotics and biocides is a growing threat to the treatment and prevention of infectious skin diseases. Photodynamic therapy (PDT) and sonodynamic therapy (SDT) offer alternative procedures in the treatment of local microbial infections. The subject of our research is the development and application of new therapeutic strategies using both therapies in a combination to increase the antimicrobial efficacy. The efficacy of the combined use with two sensitizers methylene blue (MB) and 5,10,15,20-Tetrakis(1-methyl-4-pyridinium)porphyrin tetra(ptoluenesulfonate) (TMPyP) activated by light of appropriate wavelengths and high-frequency ultrasound on *Staphylococcus aureus* (*S..aureus)* and *Escherichia coli (E. coli)* was monitored by biophysical and microbiological *in vitro* methods. The antibacterial action of MB and TMPyP was evaluated using the standard microdilution method and by subculturing bacteria to agar plates determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), respectively. Our results show that there is a significant reduction in the MIC and MBC of MB for *S. aureus* already when applying PDT at a light dose of 1x 30 J/cm^2, from concentration values of 50 μM (MIC) and 200 μM (MBC) (without application of light and ultrasound) to the value of 0.78 μM. PDT at a double light dose (2x 30 J/cm^2) reduced these concentrations to 0.195 μM, and in the case of the combined application of PDT and SDT to 0.14 μM. In *E. coli* MIC and MBC decreased from 400 μM to 100 μM or 6.25 μM for both MIC and MBC after PDT at a light dose of 1x 50 J/cm^2 or 2x 50 J/cm^2, respectively. With the addition of SDT, this value was further reduced to 1.56 μM. A similar response in a reduction of MIC and MBC after PDT and SDT was observed for TMPyP. In conclusion, MB and TMPyP show antibacterial effects in connection with PDT or at a combined application of PDT and SDT. Supported by Ministry of Health of the Czech Republic, grant nr. NU21-09-00357.

Photophysical insights of thiopurine analogues as potential photodynamic therapy agents

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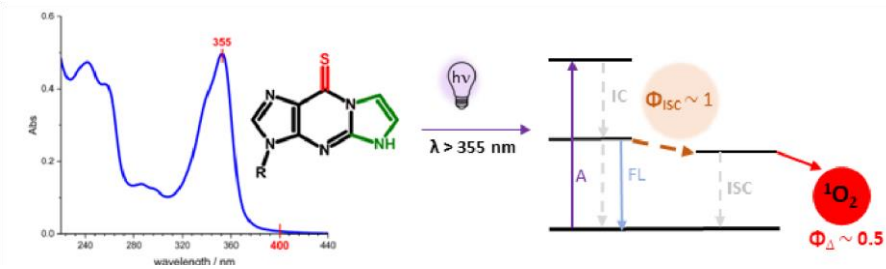
The purine scaffold constitutes the structural basis for the synthesis of its numerous analogues for therapeutic purposes[1]. The interesting biological, photophysical and physicochemical properties of the two groups of purine-based drugs i.e. thiopurines[2] and purine analogues with additional ring (1,N^2etheno analogues)[3] raised the question whether the combination of the thiocarbonyl group and the tricyclic structure will give a rise to the new group of compounds with therapeutic potential.

In this contribution, the group of 1,N^2-etheno-bridged purine analogues with exocyclic thiocarbonyl group were synthesized and their anticancer potential was evaluated on HeLa cell line. The studies on the excited singlet and triplet states of tricyclic thiopurine analogues revealed that the main channel of singlet states deactivation is intersystem crossing to the T_1 state in nearly unity quantum yield ($\Phi_{ISC} \sim 1$). Therefore, the processes which originate from the triplet excited states are the main deactivation pathways. Direct excitation of thiopurine analogues with radiation wavelengths above 355 nm results in the readily generation of singlet oxygen with yield $\Phi_{\Delta} \sim 0.5$ in acetonitrile solution. Since other thioanalogues of DNA bases have been proposed as prospective photosensitizers for the treatment of skin cancers[4], the structural and photophysical properties of thiopurine analogues with additional ring also make this group of compounds attractive candidates for phototherapy applications. In this presentation, we will discuss the photophysical, photochemical and biological properties of thiopurine analogues with additional ring to verify their potential therapeutic applications.



Photoinduced processes in thiopurine analogues with additional ring

Reference

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Photodynamic inactivation of *Pseudomonas aeruginosa* by poly-HEMA films loaded with Rose Bengal: Potentiation of the effect of potassium iodide inside and outside the polymeric matrix

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Abstract: In recent years, there has been an increase in the number of infections caused by antibioticresistant microorganisms, creating a significant social and economic problem. Various approaches have been considered to combat these microorganisms, one of which is antimicrobial photodynamic therapy. This therapy involves activating a photosensitizer with the light of an appropriate wavelength to generate reactive oxygen species [1].

For the research here presented, a series of polymeric films were synthesized, basically consisting in poly(2-hydroxyethyl methacrylate) (PHEMA) loaded with the photosensitizer Rose Bengal (RB). These polymers were characterized by UV-Vis absorption spectroscopy, Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR), and Thermogravimetric Analysis (TGA). Singlet oxygen generation was monitored by the photooxidation reaction of anthracenic probes DMA and ABDA using UVVis spectroscopy. Photodynamic therapy was conducted under different periods of irradiation with white light (LED). The iodide anion was added as an additive to enhance the efficacy of photodynamic therapy
[2][3].

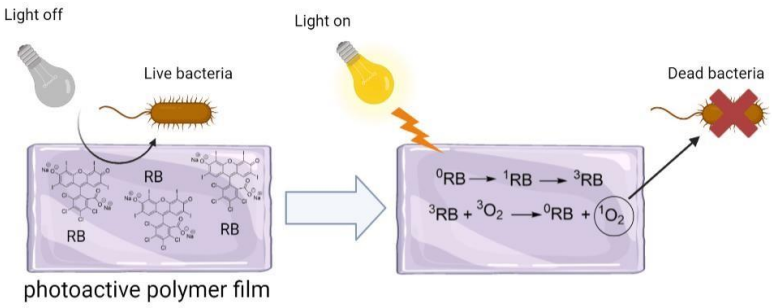

Figure 1: Schematic representation of the photodynamic action of the PHEMA films presented in this study.

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Amphiphilic cationic tripyridiniumporphyrins and their Zn(II) complexes: the influence of the irradiation wavelength and the length of the alkyl chain

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Amphiphilic porphyrins are increasingly proving to be effective photosensitizers (PSs) and promising for the use in photodynamic therapy (PDT)[1]. In our previous research, cationic porphyrins conjugated with a long alkyl chain (18C) showed much higher cytotoxicity on different cell lines compared to a hydrophilic analogue with an acetamido group[2]. Chelation with zinc(II) ion is expected to increase the lifetime of the triplet excited state (^{3}PS*) and improve singlet oxygen production[3]. Since chelation reduces the number of Q bands from four to two with negligible absorption above 610 nm, activation of Zn(II) porphyrins with red light common to PDT is unlikely to be effective, while orange light is much better suited to their optical properties, which still penetrates tissue relatively well.

We will present the preparation of free-bases and Zn(II) complexes of *N*-methylated tripyridinium-3ylporphyrins conjugated with alkyl chains of different lengths (1C, 8C-18C) and a study of the influence of their lipophilicity on PDT activity against melanoma cell lines. This study includes the determination of photophysical and photochemical properties in different solvents using various spectroscopic methods (UV-VIS and fluorescence spectroscopy, laser flash photolysis (LFP) and time-resolved fluorescence spectroscopy (TRF)) as well as *in vitro* biological analyses on two different melanoma cell lines (A375 and MeWo) and human dermal fibroblasts (HDF), where cellular uptake of each PS, its intracellular localisation and cytotoxicity were investigated. In addition, the MTT assay was used to compare the application of two different wavelengths for photoactivation of the PSs, red (645 nm; 2.0 mW / cm^2) and orange light (607 nm; 2.0 mW / cm^2).

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Rapamycin enhances Photodynamic therapy in cutaneous Squamous Cell Carcinoma

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4. *Universidad de Alcalá de Henares*

Non-melanoma skin cancer (NMSC) is the most common type of cancer worldwide classified in two types: basal cell carcinoma and squamous cell carcinoma (SCC). SCC is more aggressive, triggering severe health and aesthetic complications. For NMSC treatments, there are different non-invasive approaches as Photodynamic therapy (PDT). PDT is based on the combination of O_2, light and a photosensitizing (PS) agent. The PS, in the presence of light produces reactive oxygen species (ROS) causing cell death. PDT produces very satisfactory results in clinic, however, some cells can survive, producing tumor relapses. The combination of PDT with other treatment modalities can prevent this problem. Rapamycin is an mTORC1 inhibitor that interferes with protein synthesis and cell metabolism. In sense, we aim to study the role of Rapamycin as a neo-adjuvant therapy of PDT on SCC by using *in vitro* models.

We have evaluated effects of Rapamycin itself and in combination with Methylaminolevulinate (MAL)PDT in 2D and 3D SCC-13 cell models.The results obtained indicated that Rapamycin potentiates PDT effects, increasing cell death measured by the MTT and the the Acridine Orange / Propidium Iodide assays in 2D and 3D models, respectively. In addition, we observed an increase in the expression of active Caspase-3 and cytoplasmic localization of Cytochrome C, indicative that cell death occurs by apoptosis. We have also evaluated PpIX accumulation by flow cytometry as well as its localization. The combination of Rapamycin + MAL-PDT increase the production of PpIX in both SCC cell lines as well as ROS accumulation after cell irradiation with red light. To uncover the possible action mechanism behind the combined treatment, we have evaluated, through WB, elements of the PI3K/AKT/p70 and MAP kinases pathways.

To conclude, Rapamycin acts as an enhancer of MAL-PDT efficacy in SCC *in vitro* models, promoting higher levels of PS and ROS that leads to cell death. Several pathways as the MAP kinases are behind the molecular mechanism of this increased PDT efficacy.

Partial oxygen pressure prior and post AGuIX® nanoparticles-based PDT in glioblastoma models

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Oxygen is one of the key components of photodynamic therapy (PDT), yet severe hypoxia in tumors, especially in glioblastoma might be a major limiting factor of this treatment. Therefore, we focused on vascular changes and partial oxygen pressure (pO_2) after tumor vasculature-targeted PDT with functionalized AGuIX® nanoparticles. We investigated the oxygenation levels in murine GL261 and human U87-MG glioblastoma models *in vivo*.

U87-MG tumor were grown in Balb nude mouse legs. Effects on tumor vasculature were investigated by USG with Doppler and PW mode (VEVO 2100), electron paramagnetic resonance (EPR) oximetry. GL261 cells were implanted in the brain of the C57bl57 mice using a stereotactic device. Effects on pO_2 were investigated by MRI and EPR. AGuIX® photosensitizer containing porphyrin (synthetized by NH TherAguix SA, France) was injected i.v. at 1.75 μM/kg BW and after interval 15 min or 1h tumors were irradiated with 650 nm light at 150 J/cm^2, 160 mW/cm^2.

Tissue oxygenation level was followed over several days *in situ* in living animals by Electron Paramagnetic Resonance (Bruker Elexsys-II E540) with OxyChip, or LiBuO-derived microspheres used as a constant reporter of the local pO_2 value.

Glioblastoma tumors were extremely hypoxic in orthotopic location (pO_2 approx. 3 mm Hg) and better oxygenated in s.c. location (pO_2 approx. 10 mm Hg). Total vessel area and vessel density were substantially decreased in PDTtreated tumors, consistent with destruction of vascular structure and functionality. Fluctuation of pO_2 and blood flow reflected a loss of vessel function in tumors and surrounding tissues after PDT with AGuIX® nanoparticles.

Tumors were responding to the therapy providing that the initial vascular network was present.

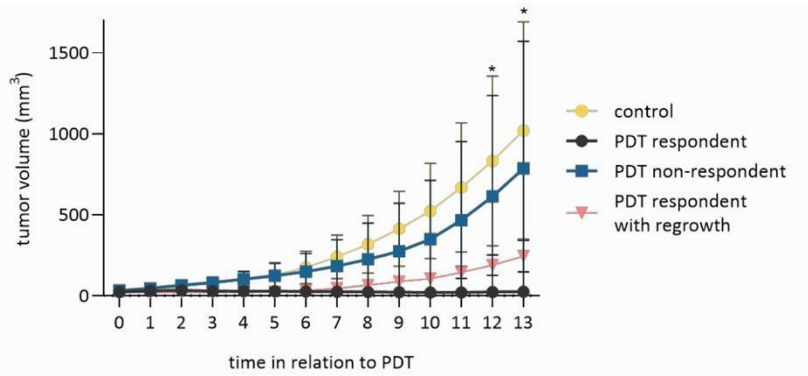

Fig. Growth curves of U87 tumors grafted ectopically in the mouse leg after PDT and control treatment, shown as responders and nonresponders. Graph represents mean with SD, statistically significant differences were found between responders vs non-responders and responders vs control at days 12 and 13 after treatment *P,0.05. N>3

Molybdenum cluster-based photosensitizers for photodynamic therapy of cancer

Tomáš Přibyl [1], Kaplan Kirakci [2], Ivana Křížová [3], Robert Pola [4], Marina Tavarez [4], Denisa Lizoňová [5], Tomáš Ruml [1], Kamil Lang [2], Jaroslav Zelenka [1]

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Photodynamic therapy, which uses photosensitizers forming reactive oxygen species after light activation, is known as a promising treatment for a variety of cancers. In recent years, the concept of X-ray-induced photodynamic therapy has aimed to overcome one of the disadvantages: the limited penetration of light into deep-seated tumors. In this work, we are investigating the nanoformulation of octahedral molybdenum clusters as a promising photosensitizer/radiosensitizer moiety on ovarian and prostate cancer cell models. Molybdenum clusters with $\{Mo_6I_8\}^{4+}$ core produce highly reactive singlet oxygen upon irradiation with a wide range of sources including blue light (460 nm) and X-rays. To evaluate the efficacy of the molybdenum clusters as photo/radiosensitizers, *in vitro* studies were conducted using ovarian and prostate cancer cells. Cells were incubated with the synthesized clusters and subjected to X-irradiation at various doses. Cell viability, apoptosis induction, and generation of reactive oxygen species were also evaluated as indicators of therapeutic efficacy. Molybdenum clusters can reduce the growth rate of cancer cells after irradiation, replacement of apical ligands in the structure can alter organelle colocalization, nanosizing increases water stability, and targeting groups can reduce toxicity to fibroblastic cell line. This work was supported by grant no. 21-16084J given by the Czech Science Foundation.

Developing a SWIR/NIR Imaging System for Singlet Oxygen Detection in Biologically Relevant Environments

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Singlet Oxygen ($^1O_2$) is the most relevant Reactive Oxygen Species (ROS), playing a fundamental role in photodynamic therapy (PDT) and antimicrobial PDT (aPDT). Great efforts have been made in developing techniques and/or methods that enable not only detection, but also quantification of the generation of $^1O_2$. It can be directly detected through its intrinsic weak phosphorescence at 1275 nm.

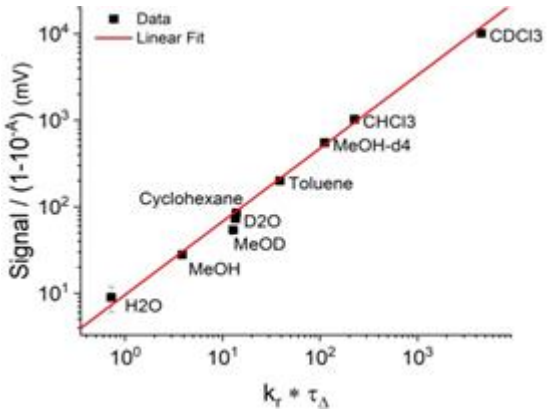

Figure 1: Validation comparing the signals with the Φ_Δ of the literature.

In the present study, a Near Short-Wave Infrared (NIRSWIR) microscope was designed and constructed for the purpose of imaging singlet oxygen, taking advantage of its weak NIR phosphorescence signature. Briefly, a continuous-wave laser is used to excite the sample, while the $^1O_2$ phosphorescence is recorded using spectral filtering with a femtowatt sensitive InGaAs photodiode. The system is validated acquiring a set of signals for phenalenone dissolved in several solvents and comparing the slope of them with the $^1O_2$ phosphorescence quantum yield available in the literature (figure 1).

Once the prototype is validated, the image is reconstructed by scanning point-by-point the sample using a 3D stage. The first preliminary images are based on the spatial characterization of the system using organic cotton fibers dyed with photosensitizers (figure 2) and small biologically relevant samples, such as plant leaves or cells incubated with different photosensitizers. Unraveling $^1O_2$ generation in biological medium will be of great help to understand the mechanisms of PDT.

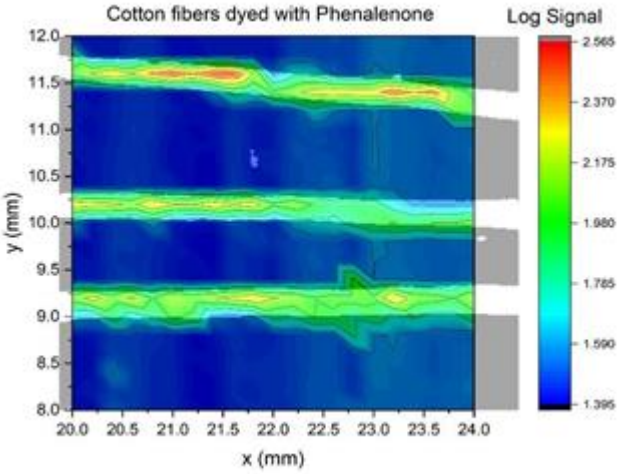

Figure 2: 4x4mm image of three cotton strings dyed with phenalenone, overlapped with a picture of the sample.

Acknowledgment: This research was funded by the Agencia Estatal de Investigación and FEDER (ref. PID2020-115801RB-C22)

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A joint experimental and computational study on the influence of packing on organic compounds luminescence and singlet oxygen generation in the condensed phase

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Since the early 2000's with the introduction by B.Z. Tang[1] of the concept of Aggregation Induced Emission (AIE), solid-state emissive organic chromophores have been employed to create new smart materials for detection in fields as varied as bioimaging,[2] mechanical constraints identification or fighting counterfeiting.[3] Recently, AIE chromophore have also emerged as possible candidates for Photodynamic Therapy (PDT).[4] For designing such devices, the Tetraphenylethelene (TPE) has been extensively used as its tremendous AIE properties are preserved even in highly functionalized derivatives. The functionalization of TPE tetrasubstituted central ethylene bond leads to stereoselectivity challenges. We recently developed a stereoselective synthesis for a new TPE related compound which demonstrates interesting AIE properties in nanoaggregates. In solution, spectroscopic and *in silico* studies revealed that fluorescence quenching proceeds with photoreactions of isomerization and cyclization. Upon further functionalization involving cationic derivatives, we hope to attain emission in the far-red and near infrared region, useful for bioimaging applications. Finally, the impact of counterion on the photosensitizing properties of the nanoobjects will be assessed.

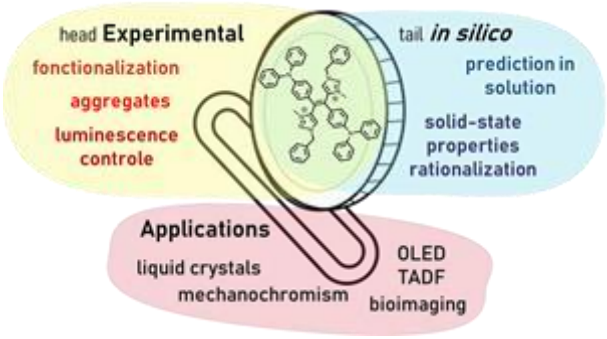

Figure 1. Dual Experimental-in silico approach to rationalize the AIE properties of target compounds

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Combining Photoluminescence and Transient Absorption Spectroscopy for Photodynamic Therapy Research

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The development of photodynamic therapy (PDT) approaches based on the generation of singlet oxygen poses challenges such as low yields of $^1O_2$ and a lack of information on the reaction mechanism. Finding new, efficient photosensitisers requires a full characterization of their triplet excited state lifetime, as well as the yield of $^1O_2$ upon photoexcitation. This presentation will introduce unique instrumentation for characterisation of PDT materials, combining spectral and time-resolved photon-counting photoluminescence in the NIR range as well as nanosecond transient absorption. This technique will be demonstrated in a variety of photosensitisers.

LP980 Spectrometer for combined transient absorption and photoluminescence measurements

Antibacterial and antiviral properties of Temoporfin and some of its congeners

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Photodynamic techniques use specific dyes - photosensitizers - that can be activated by absorption of visible light to form reactive oxygen species (prominently singlet oxygen) which in turn oxidize biomolecules and thus are capable of destroying eukaryotic as well as bacterial cells. Today, apart from the well-known application in tumour therapy (PDT) another rapidly developing field for photodynamic techniques is antimicrobial phototherapy as the increasing drug and multi-drug resistance of bacteria and microorganisms against standard antibiotics is a serious problem for public health systems around the world [1,2]. In addition, photodynamic techniques in the course of the covid-19 pandemic find growing interest for deactivating viruses [3].

As the development of new drugs is time-consuming and costly, an interesting approach is the repurposing of active substances of existing drugs for such fields of application. Having this in mind and based on literature findings we investigated antibacterial and antiviral properties of the photosensitizer Temoporfin (5,10,15,20-tetrakis(3hydroxyphenyl)chlorin) and some of its congeners. Temoporfin is the active substance in the medicinal product Foscan® which is authorized in the EU for the palliative treatment of head and neck cancer.

The antibacterial properties of the compounds have been tested against e.g., *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as examples for Gram-positive and Gram-negative germs, respectively. The antiviral activity was investigated with HIV and SARS-CoV-2 pseudoviruses.

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A case of Photodynamic therapy for in-situ carcinoma of esophagus in a patient with inoperable condition.

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Introduction; Although surgical resection is the best way for curing cancer, there has been a certain portion of patients who could not undergo surgery because of various reasons. Recently we experienced one case of photodynamic therapy (PDT) for "in situ carcinoma" of esophagus in a patient with inoperable condition, and investigated the feasibility and safety of photodynamic therapy.

Case and treatment results; Medical records of an 86 years old male patient who underwent PDT for his esophageal cancer (squamous cell carcinoma in situ, located in 32cm from incisor) in 2022. He had suffered from lung as well as bladder cancer in 2020, and therefore hard to undergo another surgery for his new malignancy. Photosensitizer (Photofrin) was administered intravenously 48 hours before starting PDT. Two days after the 1st PDT (total energy of 160J, with cylindrical probe) 2nd PDT was performed (total energy of 140J). Examination using gastrofiberscopy was performed at 1-month, 2-month and 6month after PDT. The patient was in recurrence free status until last follow-up.

Conclusion; Photodynamic therapy could be an option of curative therapy for early esophageal cancer, especially for patients with poor general condition or refusal of surgery.

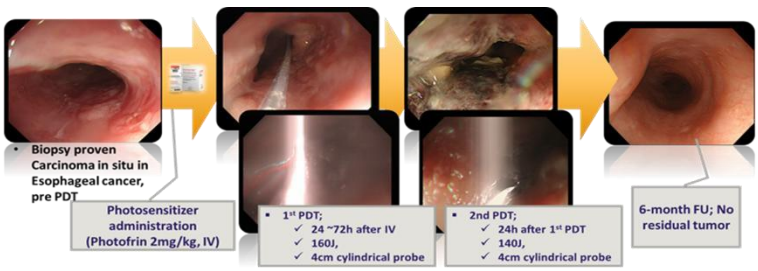

Figure 1; Flow diagram of PDT in esophageal cancer

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Photoprotection, skin photobiology and health

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The dual protective role of trans-cinnamic acid as an antioxidant and iron chelator in UVA-irradiated skin fibroblasts

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Exposure to skin fibroblasts to solar UVA radiation results in excess generation of reactive oxygen species (ROS) and the release of potentially harmful labile iron (LI), thereby causing oxidative damage to cell components which if remained unrepaired, can lead to necrotic cell death. The pre-treatment of cells with synthetic iron chelators has been shown to be protective against UVA-induced cellular damage by depleting the intracellular labile iron pool (LIP) in skin cells [1]. With the trend of consumer market towards the sole use of natural products for skincare and photoprotection, it has been a necessity to identify natural antioxidants that may possess iron-chelating properties in addition to their antioxidant properties. This will allow to both sequester free radicals and adjust excess free LI released after exposure of cells to damaging doses of UVA component of sunlight [1]. Trans-cinnamic acid (t-CA) is the primary form of cinnamic acid that is present mainly in the bark of *Cinnamomum cassia*. It is widely recognized for its antioxidant properties by scavenging free radicals and has demonstrated potential benefits in skin photoprotection against UVA radiation [2]. However, no research has been conducted on its iron chelating properties. The present study aimed to assess the photoprotective efficacy of this natural compound against UVA-induced oxidative damage in cultured human primary skin fibroblasts by assessing its ability to chelate intracellular LI. Cultured fibroblasts were treated with the t-CA compound at a series of concentrations up to 40 μM for 24 hours. These treatments demonstrated a significant reduction in cytosolic LIP as assessed by our recently developed highly sensitive fluorescent iron sensor. The reduction in cytosolic LIP was also observed after UVA radiation and this correlated with photoprotective role of tCA up to a high UVA dose of 500 kJ/m^2, when compared to untreated and unirradiated control fibroblasts. Our results suggest that t-CA exhibits a promising potential for protecting the skin against the harmful impact of UVA radiation by mitigating both the basal and UVA-induced increase in cytosolic LIP in skin cells and thereby preventing the radiation-mediated oxidative damage. This can be attributed to its dual function as an antioxidant and iron chelator.

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Photoprotection of animals: Livestock shade cloths for free-range pigs

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Background: Nowadays, pig breeds do not have fur. Their skin sensitivity to ultraviolet radiation (UVR) is similar to that of light-skinned humans. For piglets, erythema may become life-threatening, often leading to death. To protect animals from the sun, livestock shade cloths are in use. The main application is to provide a shelter and shading animals from solar radiation heat. The shading ability is reported by the „Shading Rate“, which is the percentage of visible light passing through the shade cloth. However, no information is available on the potential protection from UVR. The aim of this study was to evaluate the effectiveness of shading cloths against erythemally weighted solar UV radiation.

Material and Methods: In a first step, the UPF (ultraviolet protection factor) of different shading cloths (shading rate: 50%, 75% and 90%) was tested in the laboratory according to the test procedure of human clothes. In practice, the shadow cloth is mounted horizontally at a height of around 2 m allowing access for agricultural workers. Therefore, mainly diffuse UVR reaches the animal's body from several directions. To estimate the UVR protection, more than 20 electronic UVR-meters (type Sunsaver) were mounted on a true-to-life pig model, positioned on a rotating platform. Measurements were taken in the blazing sun and under different shading cloths.

Results: Measurements show that shade cloths can protect pigs effectively from solar UVR. Protection varies depending on the UPF of the shade cloth, its size, and the mounting height. The protection factor also depends on the body site and varies with solar elevation. Most advantageous is the application for those body parts which may directly face the solar beam and the circumsolar disk. Such parts can receive exposure higher than ambient UV radiation (measured horizontally).

Conclusion: Free-range pigs can be effectively protected from solar UV radiation by shade cloths. Protection factors are in the range of those known from shade structures used for humans (sunblind, etc.). Especially effective is the protection from direct solar beam. However, shade structures can substitute neither the primordial fur, nor the possibility to wallow in mud and should be the last measure.

DNICs exposure to radiation induces nitric oxide release

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Insufficient exposure to sunlight is considered a public health problem, being correlated to different diseases, such as the incidence of breast cancer, hypertension, cardiovascular disease, metabolic syndrome, multiple sclerosis, type 1 diabetes, myopia, among others. It is suggested that such conditions may be related to the ability of the epidermis to release nitric oxide(NO) after exposure to sunlight, with subsequent local and systemic effects , such as neutralizing reactive oxygen species and decreasing blood pressure, respectively.[1,2,3] Recently, the velocity of NO release after exposure to UVA radiation has correlated with the possible ability of the skin to maintain NO stores .[4,5] DNICs (dinitrosyl iron complexes) are known to be important NO stores in tissues, and epidermis is an important route of iron release from our organism and it has a measurable impact on systemic iron metabolism. [6,7] The relationship between the concentration and release of iron in the skin may be related to the local concentration of DNICs, but this connection has never been properly investigated.[8,9] In this work we exploited the possible role of DNICs being the molecular complexes that can release NO upon absorption of sunlight. DNICs were synthesized and irradiated (in the 45-70-90 µM range) with UVA radiation (365nm, 12-25 joules) and blue/violet light (410nm,25-50 joules). In order to evaluate the light-dependent degradation, we analyzed the absorbance of the synthesized compound in dark and submitted to irradiation. We also measured the levels of nitrite generated by the Griess test,which indirectly infer the levels of nitric oxide produced. Our results showed a dosedependent degradation after UVA and blue/violet light irradiation. DNICs also release nitric oxide in a dose-dependent manner after irradiation. Such results are important because they demonstrate that such NO stocks, possibly present in the skin, can act as NO donors during exposure to radiation and, consequently, have corresponding effects related to the concentration of iron existing in the tissue.

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Photoprotection by sheer tights in-situ

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Background: Pantyhose, or "sheer tights", are frequently worn elastic garments, which conform to the shape of the legs. The sun protection factor of elastic garments depends on the material's degree of stretch, which differs by body site, such as along the calf, but also by movement (e.g. knee). Additionally, body measurements (e.g. thigh circumference) vary by individual, as does the resulting stretch. A sun protection factor derived for a certain static body and a certain site may therefore have restricted validity. The aim of this study was to determine an in-situ (in-person, in motion) sun protection factor considering this variability.

Material and Method: For this study, a questionnaire was done to gain realistic circumferences (thigh, knee, calf, instep) in dependence of tights size (S, M, L,…), body height, and weight. Different tights (varying in DEN, colour, material) were stretched according the gained measures, and an erythemally weighted ultraviolet (UV) protection factor was determined. To gain the in-situ protection factor, a volunteer was equipped with 10 electronic UV-meters located under and above the pantyhose at different body sites (thigh, knee, calf, shin, instep). The postures used were walking (towards the sun, away from the sun, in a circle) and seated facing the sun.

Results: Measurements show that the erythemally weighted UV protection factor differs by density (DEN), colour, and material. The differences in UPF are most pronounced for different DEN, and therefore also for different degrees of stretch. This is followed by differences in colour, such as beige vs. black, which can constitute up to around 50% increase in UPF. The material has the least impact on UPF. A variation of UPF along the legs can be seen due to the varying degree of stretch, with the effect more strongly pronounced for higher DEN. Naturally, differing UPF also results from individual circumferences or height (at same tights size), thus from respective weight and Body Mass Index.

Conclusion: Our study shows that the sun protection factor in-situ is difficult to estimate, as it is influenced by several factors. Using the European standard method for determining UPF in clothing (EN 13758) produces a maximum UPF that is not reached in-situ. However, it would be possible to determine a minimum (90% confidence interval) protection factor by taking into account real body measurements.

Photon and singlet oxygen induced cis-trans isomerization of the water-soluble carotenoid crocin.

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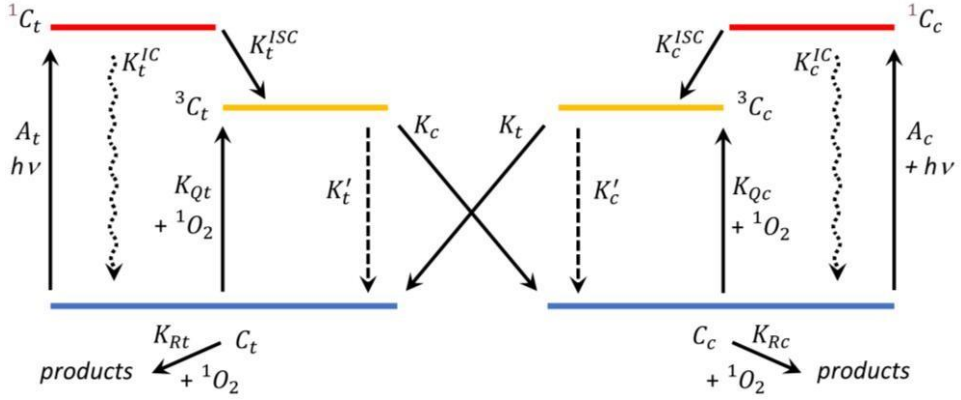
Studying the cis-trans isomerization process in crocin (CR), one of the few water-soluble carotenoids extracted from saffron, is important to better understand the physiological role of ciscarotenoids *in vivo* and their potential as antioxidants in therapeutic applications. For that, cis-trans isomerization of both methanol- and water-dissolved CR was induced by light or thermally generated singlet oxygen ($^1O_2$). The kinetics of molecular concentrations were monitored by both HPLC and nondestructive spectrophotometric methods. These last allowed to simultaneously follow the cis-trans isomerization, the possible bleaching of compounds and the amount of thermally-generated $^1O_2$. Our results were in accordance with a comprehensive model where the cis-trans isomerization occurs as relaxation from the triplet state of all-trans- or 13-cis-CR, whatever is the way to populate the CR triplet state, either by photon or $^1O_2$ energy transfer.



Scheme of the main photochemical and $^1O_2$-induced trans cis isomerization processes in crocin. A_t and A_c are the excitation rate constants from the steady state of all-trans-CR (C_t) and $13-cis-CR$ (C_c), respectively, to their singlet excited states, ^1C_t and ^1C_c, respectively. The K_t^{IC} and K_c^{IC} are the rate constants for the internal conversion from singlet excited- to ground-states; the K_t^{ISC} and K_c^{ISC} are the rate constants for the intersystem crossing to generates the triplet energy states, ^3C_t and ^3C_c of C_t and C_c, respectively. $K_{Q_{t,c}}$ and $K_{R_{t,c}}$ are the bimolecular rate constants for physical and chemical quenching of $^1O_2$, respectively. The deexcitation rates of the CR triplet states are indicated by K_t and K_c for the trans↔cis isomerization processes and by K_t' and K_c' for the internal relaxation processes.

The process is much more (5 to 10-folds) efficient from cis to trans that vice versa. In H_2O, a $^1O_2$induced bleaching effect on the starting CR was not negligible. However, the CR "flip-flop" isomerization reaction could still occur, suggesting that this process can represent an efficient mechanism for quenching of ROS *in vivo*, with a limited need of carotenoid regeneration.

Acknowledgment

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Photoprotection by hand-held sun umbrellas

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Background: The WHO recommends the application of sun protection measures above a certain threshold of erythemally effective irradiance. Such sun protection could be headwear, long sleeved garments, sunscreen, or seeking shade. Hand-held sun umbrellas are in use in Asia as protection against UV radiation and heat. In the past, hand-held umbrellas have also been used as sun protection in western populations to maintain noble paleness. There is a certain potential for comeback. However, little information is available on the effective sun protection capabilities of hand-held umbrellas. This study aims at evaluating the sun protection factor of hand-held sun umbrellas.

Material and Method: Different handheld umbrellas were examined in respect to their ability to protect against erythemally effective UV radiation. The examined pieces differed in material (oiled paper, silk, cotton, cotton lace, synthetics) and diameter (20" to 68"). The UPF of the material was tested in the laboratory. As the UPF is not an accurate measure for sun protection of a person, volunteers carrying umbrellas while walking (towards the sun, away from the sun, random walk) were equipped with electronic UV-meters on the upper part of the body. Three different types of carrying positions were used (horizontally, directed towards the sun, shouldered).

Results: Our measurements show that at least a person's head can be protected by a sun umbrella. Nevertheless, the lack of all-around protection and consequent diffuse UVR result in a rather low protection factor compared to clothes or sunscreen. The protective effect depends on the size of the umbrella and solar elevation, as well as on the material: due to the number of perforations in the material, cotton lace was shown to offer the lowest protection, well below the UPF of most sunscreens. Silk, oiled paper, and cotton each had a higher UPF, from low to high respectively, with 210T-Pongee (a 100% Polyester, UPF 50+ certified material) ranking as the most effective of the examined materials. The umbrella position and orientation relative to sun and body also factor in to the results: most effective but most inconvenient is carrying it towards the sun. Horizontal orientation shows good protection in any direction. Shouldering, the most convenient way, is least effective on average.

Conclusion: Our study has shown that hand held sun umbrellas can protect against erythemally effective UV exposure to a certain extent, especially providing protection for vulnerable areas generally not covered by summer street wear, such as head and shoulders, and can prolong the time outdoors without sunburn.

Design and evaluation of novel UV filters for photoprotection

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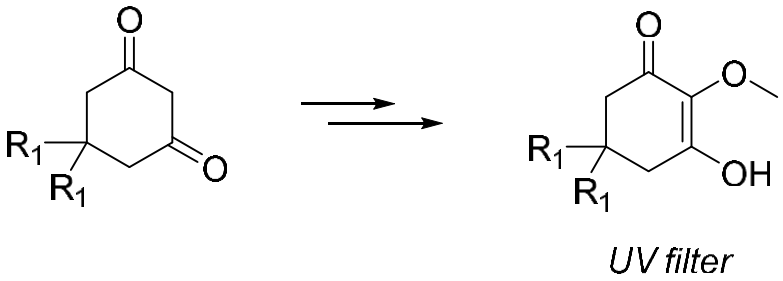
Solar radiation can lead to various harmful effects, including cancer and death, through several photochemical and photobiological reactions.[1]

UV radiation, specifically UVA and UVB (280-400 nm), is responsible for skin damage. Current commercial sunscreens are able to minimize these effects, but they also present relevant drawbacks and limitations. To address this issue, there is a need to develop new UV filters that possess appropriate photoprotecting capabilities for human use, including high stability, biodegradability, efficient energy dissipation mechanism, and strong UV absorption.[2]

In this study, we synthesized a diverse set of UV filters based on the structure of the natural molecule gadusol and evaluated their photoprotective properties using computational methods, optical methods and photostability studies. Our results indicate that these synthetic UV filters show promise in protecting different surfaces, including human skin, and can potentially reduce the harmful effects of solar radiation.



Scheme 1. Synthesis of UV filters

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Use of reconstructed skin model to demonstrate the photo-protection afforded by highly photoprotective sunscreen products-against DNA lesions and cellular alterations

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Introduction: Protecting the skin from UV rays is a public health issue. Both UVB and UVA rays induce multiple damages in the epidermis and the dermis that contribute to photo-carcinogenesis and photoageing. Sunscreen products are used to prevent these deleterious effects. In the present study, the level of photoprotection afforded by 2 highly protective sunscreens products : one with SPF50+ and a very high UVA protection with a new long UVA filter filtering between 380 and 400 nm) and 1 ISO sunscreen standard P6 (SPF40) were analyzed and compared by using UV Solar Simulated Radiation (UVSSR)-induced damages on reconstructed skin tissues. The photoprotective potential against UVSSR exposure was evaluated according to several biological parameters, including DNA lesions.

Method: The T-Skin^{TM} model (EPISKIN) is composed of a fibroblasts populated dermal equivalent and a fully differentiated epidermis. Tissues were treated with sunscreens (1,3mg/cm² topically applied on PMMA plate) and then exposed to UVSSR doses (0, 5 and 40 J/cm²) using an Oriel 1600W 3A solar simulator equipped with WG325 + UG11 filters. Twenty-four hours after exposure, tissues were analyzed for skin viability and morphology using Hemalun-Eosin-Saffron (HES) staining and DNA lesions formation using CPD immunolabelling and quantification. The levels of damage were analyzed for the 2 tested formula and compared to those of untreated tissues exposed at the same UVSSR doses.

Results: For untreated-tissues, UVSSR exposures induce a decrease in epidermal and dermal viabilities , associated with morphological damages as soon as 5J/cm^2. These damages were amplified in a dose dependent manner. At the dose of 5J/cm², viability and morphology were not modified in all sunscreenprotected samples compared to non exposed samples. At 40 J/cm^2, skin epidermal viability decreased and morphological damages were observed for formula P6 whereas the sunscreen with very long UVA protection still protected the samples leading in the latter cases to tissues comparable to unexposed tissues. As soon as 5J/cm², DNA lesions were detected in all cell nulei in unprotected samples. In the sunscreen-protected samples, the protection against DNA lesions formation was still complete for the very long UVA protection sunscreen even at 40J/cm² while at that dose CPD+ cells could be detected in the P6 condition. The higher protection of the sunscreen with very long UVA protection was statistically significant compared to that of P6.

Conclusions: This present study shows that the efficacy of photoprotection afforded by sunscreens formula can be discriminated on reconstructed skin tissues (T-Skin^{TM} model) regarding UVSSR induced skin damages, especially DNA lesions. The data obtained with the reference formula P6 and the sunscreen with very long UVA protection are in agreement with their SPF values, as determined by the ISO 24 444 clinical SPF method. The model allowed to demonstrate the higher efficacy of the sunscreen with very long UVA protection up to the highest UVSSR dose compared P6 formula. This is of great importance considering the mutagenic potential of such DNA lesions.

Antioxidant properties of vanillin during photosensitized oxidation of biomolecules

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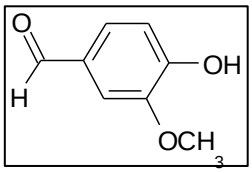
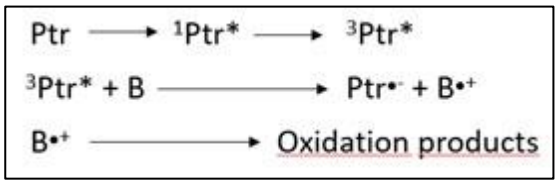
Vanilla is a popular extract from mature pods of the orchid *Vanilla planifolia*. 4-hydroxy-3methoxybenzaldehyde, known as vanillin (VAN) is the molecule that gives the extract its aromatic properties. It has been reported that VAN has anticancer, antioxidant, anti-inflammatory, neuroprotective properties among others.[1]



However, not much is known about the antioxidant ability of VAN in radiation mediated processes. For this reason, the main goal of this work is to evaluate if VAN can prevent the photosensitized oxidation of biomolecules.

Biomolecules (B) are oxidized by both direct and indirect absorption of electromagnetic radiation. UV-A radiation (310-400 nm) is about of 95 % of the total UV radiation of the sun reaching the earth surface, and, in general, its poorly absorbed by B. However, UV-A degrades B through photosensitized mechanisms.[2] Pterins (Ptr) are natural compounds, present in all living systems, that can be accumulated in human skin during pathological conditions. It has been demonstrated that, under UV-A radiation, Ptr are capable to photosensitize the oxidation of B like proteins, DNA, and their components.[3] Ptrphotosensitized degradation of B is mainly a type I mechanism and is initiated with an electron transfer from B to triplet excited state of Ptr.



Herein, we report the efficiency of vanillin to reduce the degradation of a nucleotide (2´-deoxyguanosine 5´monophosphate, dGMP) and an amino acid (tryptophan, Trp) during UV-A irradiation in the presence of Ptr. To carry out this study, aqueous solutions of dGMP or Trp, and Ptr (pH 6, room temperature) were exposed to UV-A radiation (λ_{exc}=365 nm) during different times in presence and absence of VAN. The photochemical reaction was studied by UV-Vis spectrophotometry, HPLC, fluorescence spectroscopy. Results indicate that the photoinduced damage of B is reduced in the presence of VAN. The mechanistic study indicates that in our experimental conditions the inhibition of the photosensitized process is due to the deactivation of B radicals.

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Characterization of new photo-protective agents from *Cyclopia spp.* (Honeybush) as bioactive UV filters for skin protection

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Skin photoaging is premature aging of the skin caused by repeated exposure to ultraviolet (UV) rays. UVA rays (320-400 nm) are capable of penetrating deep into the epidermis and their impact is currently acknowledged as a determinant co-activator in skin photoaging and the development of skin cancer. Indeed, UVA radiation induces a range of physiological photoaging reactions, including chronic inflammatory signalling, dermal matrix degradation, solar elastosis and alteration of tissue homeostasis [1]. Among the various defence measures adopted to reduce the damage induced by overexposure to sunlight, the most common is the use of sunscreens that shield UVR due to the presence of chemical and/or physical UV filters. It has been reported that some UV filters, such as benzophenone-3 (BP-3), may lead to adverse effects, toxicity for human health and even ecological risks [2]. Thus, there is a growing tendency to introduce environmentally-friendly products in the cosmetic market focused on using natural compounds or plant extracts due to their reported photoprotective capacity. Honeybush (HB) (*Cyclopia spp.*) is an endemic plant of South Africa known for its high polyphenol content and its countless beneficial properties. The phenolic profile varies qualitatively and quantitatively depending on the species, but the main constituents belong to the subclasses of xanthone, benzophenones, flavanones, flavones and dihydrocalcones [3].

We here report an investigation on the potential of honeybush polyphenols, in particular benzophenones, as photoprotective agents which could partially substitute synthetic UV filters, such as BP-3. The spectral profiles of a honeybush extract, a benzophone-enriched fraction and an isolated benzophenone diglucoside show that they broadly absorb in the UVB/UVA range and that they are able to protect against UVA-induced damage to proteins (Fig. 1a), lipids (Fig. 1b) and human dermal fibroblasts (Figs. 2a-2b), suggestive of their possible use as new, naturally-derived, UV filters.

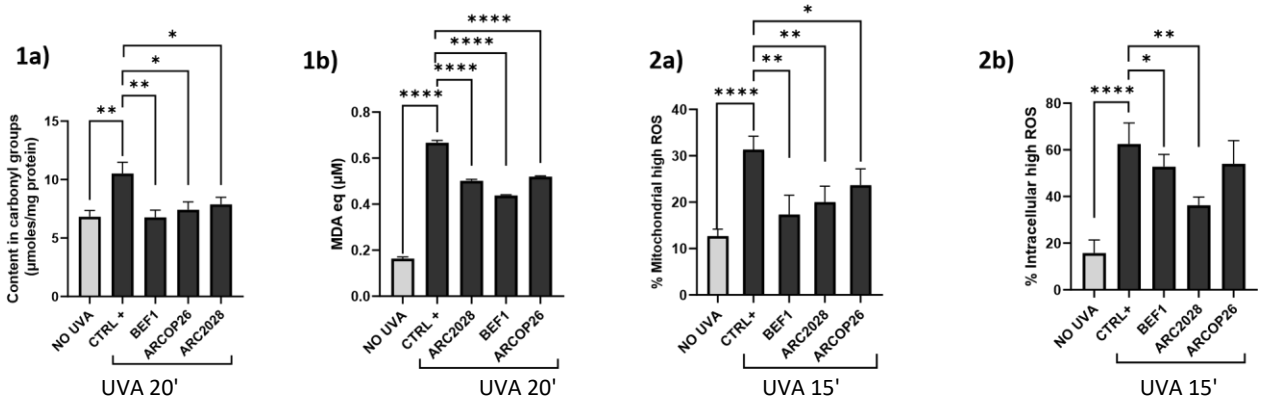

Figure 1: Oxidation damage in the presence or absence of honeybush extract (ARC2028), benzophenone-enriched fraction (BEF1) and an iriflophenone diglucoside (ARCOP26) used as shielding agents, in BSA **(a)** and PC liposomes **(b)** after 20 min UV irradiation (One-way ANOVA: ****p<0.0001; **p<0.001; *p<0.05 vs CTRL+).

Figure 2: Production of mitochondrial **(a)** and intracellular **(b)** high ROS (expressed as a percentage) in Human Dermal Fibroblasts in the presence or absence of honeybush extract (ARC2028), benzophenone-enriched fraction (BEF1) and an iriflophenone diglucoside (ARCOP26) used as shielding agents after 15 min UVA irradiation (One-way ANOVA: ****p<0.0001; **p<0.01; *p<0.05 vs CTRL+).

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Insights on the photoprotective potential of marine-inspired thiol compounds

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Despite the knowledge that sunscreens are an undeniably important tool in the fight against skin cancer, their formulations may need to be improved to contain safer ingredients, particularly regarding the concerns raised on the potential eco-toxicity of some sunscreens, containing benzophenone-3 (oxybenzone) and octyl methoxycinnamate (octinoxate), that have now been banned from sale and distribution in Hawaii (and other USA locations) [1].

Marine organisms living in the photic zone of the sea are constantly exposed to changes in light intensity and spectral composition; for this reason, they have evolved the ability to use solar radiation for survival but also to protect themselves from UVR-induced damage. Such an environmental constraint has forced them to evolve a complex system of photoprotective mechanisms, antioxidant enzymes and molecules, in order to counteract light-dependent stress.

Ovothiols (5-thiohistidine derivatives) are characterized by a methyl group on the imidazole ring of histidine (Fig. 1a) and are naturally present in marine invertebrates, bacteria and protists [2,3]. Thanks to the position of the thiol group on the imidazole ring of histidine, these compounds exhibit unusual antioxidant properties [2].

The aim of this study was to examine the possible photoprotective properties of these compounds (Fig. 1a). We report the absorption spectra of iso-ovothiol A (Fig. 1b) and its precursor 5-thiohistidine (Fig. 1c) before and after UVA irradiation as well as their potential photoprotective effect, in terms of screening agents on different biological macromolecules: bovine serum albumin (BSA) (Fig.2a) and phosphatidylcholine (PC) liposomes (Fig. 2b). These preliminary results show that these compounds may protect against UVA-induced oxidative damage.

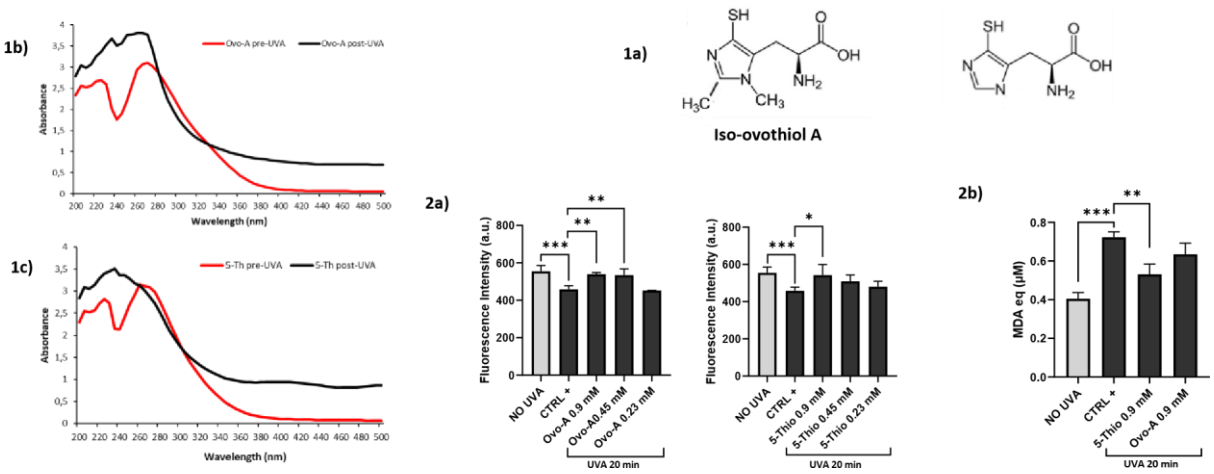

Figure 1: Chemical structure of the reduced forms of the two marine compounds **(a)**; absorption spectra of 5-thiohistidine (0.9 mM) **(c)** and iso-ovothiol A (0.9 mM) **(b)** before (red) and after (black) 20 min UVA irradiation (54 J/cm^2).

Figure 2: Oxidation damage in BSA **(a)** and in PC liposomes **(b)** in the presence or absence of 5-thiohistidine and iso-ovothiol A at different concentrations used as shielding agents, exposed to 20 min UVA irradiation (54 J/cm^2) (one-way ANOVA: ***p<0.0005; **p<0.001; *p<0.05 vs CTRL+).

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Effect of antifungal photodynamic therapy in endodontics

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Fungal infections are usually the main cause of unsuccessful endodontic treatments. The aim of study was to evaluate, *in vitro*, the effect of photodynamic therapy (PDT) in endodontic treatment. Thirty-six bovine roots were instrumented, in a mechanized way, contaminated with *Candida albicans* (ATCC 10231) and divided into 4 groups: Control Group (CG; n=9): irrigation with saline solution; Hypochlorite Group (HG; n=9): irrigation with 1% hypochlorite solution (5 min); Chlorhexidine Group (CHX G; n=9): irrigation with 0.2% chlorhexidine (5 min); PDT Group - PIT 3 min Group (n=9); PDT Group - PIT 5 min (n=9). The PDT were mediated by methylene blue (0,005%) and low power laser (Laser Duo, MMO, São Carlos, Brasil, l=660 nm; P=100 mW; E=18 J; t=180 s). In Microbiological samples were obtained before (T1) and immediately after (T2) the interventions. Microbial reduction values were analyzed by Kruskal Wallis Statistical tests and Dunn's test, as post hoc ($p<0.05$). The results showed total eradication of microorganisms in all groups treated ($p<0.05$) (Figure 1). We can conclude that the use of agents chemical or photodynamic therapy, in the parameters tested in this study, were effective in fungal eradication of root canals.

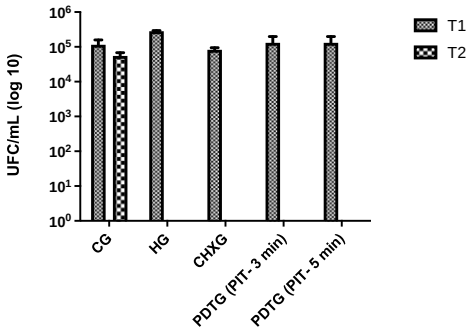

Figure 1. Microbial reduction between evaluations before and immediately after different interventions, T1 and T2 – respectively (the bars indicate the deviation standard).

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Efficacy of an enlarged photoprotection covering the whole UV spectrum: evaluation of a 1 month-clinical changes in pigmentation and wrinkles visibility through a real life split-face study

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Introduction: The current sunscreens can efficiently filter UV-wavelengths up to 370/380 nm but have limited absorption in the 370/380–400 nm range. Recently, a new cyclic merocyanine UVA1 absorber, Methoxypropylamino Cyclohexenylidene
Ethoxyethylcyanoacetate (MCE), exhibiting a maximal peak of absorption at 385 nm has been developed and was further approved by the scientific Committee on Consumer Safety (SCSS) for use. Formulations containing MCE have demonstrated a higher UVA1 protection *in vitro* and anti-pigmentation efficacy *in vivo* under controlled UV exposure.

Objectives: To evaluate *in vivo* anti-aging benefits of this broader UVA1 protection with the daily application of a sunscreen enriched with MCE for one month.

Methods: A double-blind, split half-face clinical study was conducted in Brazil during summer season with healthy females (35–65y) phototypes I to III. After 2 weeks of wash-out, a sunscreen with 1% MCE (SPF 50+) and a reference sunscreen (SPF 50+ without MCE) were applied twice daily with controlled applications for one month. Volunteers were sun-exposed up to two hours daily and had standard pictures acquisition. Dermatologists graded the pictures at baseline and after one month of applications based on reference standardized scales of Skin Aging Atlas.

Results: Clinical assessment on pictures showed that, after one month, the MCE-enriched sunscreen significantly improved wrinkles (crow's feet, upper-lip, ptosis wrinkles), texture of the mouth contour, upper-lip texture, whole face pigmentation and vascular disorders when compared to baseline and to the reference formula. In addition, MCE sunscreen presented significantly better results vs the reference for other pigmentation signals (forehead, lateral facial and upper-lip pigmentation).

Conclusions: For the first time, in real life conditions, a broad spectrum photoprotection including long-UVA provided by the MCE UV filter, is shown offering an added efficacy in the prevention and correction of facial skin aging signs of Brazilian women with Phototypes I to
III.

Enlarged photoprotection efficiently covering the whole UV spectrum: evaluation of 2 month-clinical changes in pigmentation and wrinkles visibility through a real life splitface study in different phototypes from Brazil and China.

F. Flament[1]; B. Muller[1]; D.G. Mercurio[2]; A. Li[3]; C. Tricaud[1]; F. Bernerd[4]; A.L. Demessant[5]; C. Le Floc'h[5]; N. Jager-Lezer[6]; C. Delaunay[6]

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[6] L'Oréal Research and Innovation, Chevilly-Larue, France.

Introduction: Today, state-of-the-art sunscreen formulas can efficiently filter UV wavelengths up to 370/380 nm but have limited absorption in the 370/380–400 nm range of wavelengths. Recently, a new cyclic merocyanine UVA1 absorber, Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate (MCE), exhibiting a maximal peak of absorption at 385 nm was approved by the Scientific Committee on Consumer Safety (SCSS) for use in sunscreen products.

Objectives: To evaluate in vivo global anti-aging benefits of a higher and broader UVA1 protection with the daily application of a sunscreen formulation enriched with MCE in Brazilian and Chinese populations.

Materials and Methods: A double-blind, split half-face clinical study was conducted during summer season with healthy female volunteers (30–65y) in Brazil (52 volunteers, phototypes II-III) and China (61, III-IV). After 2 weeks of wash-out, a sunscreen with 3% MCE (SPF 50+) and a reference sunscreen (SPF 50+ without MCE) were applied twice daily with controlled applications for two months. Volunteers were sun-exposed up to two hours daily and had standard pictures acquisition. Dermatologists graded the pictures at baseline and after two months of treatment based on reference standardized scales of Skin Aging Atlas.

Results: Clinical assessment on pictures showed that after two months, the MCE-enriched sunscreen significantly ($p<0.05$) improved aging signs when compared to the reference formula: i) wrinkles/texture in Brazil (Forehead, Crow's feet, upper-lip or Nasolabial) and China (Upper-lip, Texture of mouth, Nasolabial) and ii) pigmentation signs in Brazil (Whole face pigmentation and density of dark spots) and China (Contrast and Size of dark spots, Malar pigmentation, Whole face pigmentation).

Conclusions: For the first time, in real-life conditions, a broad spectrum photoprotection including longUVA, demonstrated an extra-efficacy in the prevention and correction of facial skin aging signs of Brazilian and Chinese women.

Effects of UVC light and chemical decontamination on the dimensional stability of dental impression material.

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Decontamination of dental impression materials is essential to prevent cross-infection in dental offices. This study evaluated the effects of UVC light and chemical decontamination on the dimensional stability of hydrocolloid dental impression material (HDIM). Dental mannequins were impressed with HDIM (alginate) (n = 25) and divided (n = 5): G1- control- C (no treatment), G2- 2% glutaraldehyde- GLU (10 min), G3- 1% sodium hypochlorite- HS (10 min), G4- UVC light- UVC (λ = 254 nm) (30 s), G5- Autoclave- AUT (15 min). Measurements were performed on plaster models from HDIM, with a digital caliper on the upper first molar: mesio-distal (MD-O) and buccal-palatal (BP-O) on occlusal face, cervical-occlusal (CO-B) and mesio-distal (MD-B) on buccal face. The data were analyzed using Kolmogorov-Smirnov, ANOVA one-way and Tukey tests ($p < 0.05$). There were no significant differences in the linear values (mm) of models between Control group and UVC light and chemical disinfection methods ($p > 0.05$), the autoclave showed higher distortion comparing to other groups ($p < 0.05$). The decontamination with portable UVC light equipment maintained the dimensional stability of the hydrocolloid, being an effective decontamination method, ecological, without the generation of toxic residues, viable, alternative to chemical methods.

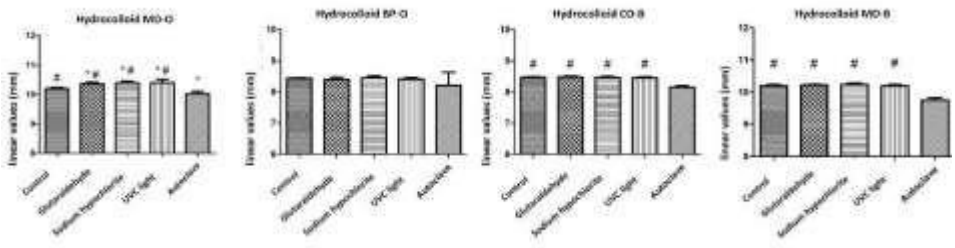

*Figure 1. Values of linear measurements (mm) of models from hydrocolloid impressions of different disinfection method (*p< 0.05 compared to control #p< 0.05 compared to autoclave NS p> 0.05 in comparisons between groups)*

Reference

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A randomized comparative study on melasma during summer with a visible light-protected tinted sunscreen versus a standard non-tinted sunscreen

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Introduction & objectives: Melasma is a common hyperpigmentation skin disorder, characterized by relapse due to sun exposure. Ultraviolet (UV) radiation is the main cause of skin pigmentation, but more recently visible light has been shown to be an important contributor. Therefore, sunscreens against UVA, UVB and visible light are key in melasma prevention, but few comparative studies have been conducted. The aim of the study is to compare a sunscreen containing photoprotection against visible light (tinted product) to a non-tinted sunscreen in the prevention of melasma relapse, using instrumental and clinical.

Materials and Method: In a single-center, randomized, investigator-blinded clinical study, 42 melasma women (mean age 39.5 years old) were included during summer with type III (93%) and IV (7%) phototypes. Divided into two groups, they applied on the whole face at least twice daily either the tinted sunscreen (SPF50+, UVA index 38, visible-light protection factor 66%) or the same sunscreen but nontinted. At 3 visits (day [D]1, T2.5 months and T5 months), melasma was assessed by colorimetric measurements using the ITA° angle (which includes the L* and b* parameters) to evaluate skin pigmentation, L parameter for lightness and ΔE calculation (which includes the L*, b* and a* values) for the color homogeneity, in comparison with the uninvolved area. Moreover, melasma was clinically evaluated using the mMASI (modified Melasma Area and Severity Index). The tolerance evaluation of the two sunscreens and the subjective efficacy were also performed at the end of the study.

Results: The difference between melasma ITA° and the uninvolved area ITA° (ΔITA°) was significantly better of 18.1% ($p<0.05$) in the tinted sunscreen group compared to the non-tinted group, confirming an improvement of skin pigmentation with the tinted sunscreen, after 5 months of use. Similarly, the ΔL between areas, and ΔE, were better than the non-tinted sunscreen of 16.3% ($p<0.05$) and 4.3% ($p<0.05$), respectively. A significant improvement was also observed in the tinted sunscreen group regarding skin pigmentation of 35.7% ($p<0.001$), skin lightness of 32.6% ($p<0.001$), and color homogeneity of 25% ($p<0.001$) at T5 months, when compared to baseline. Furthermore, the women who applied the tinted sunscreen had a significant decrease of the mMASI score of 12.5% ($p<0.001$) after 5 months, but not statistically significant when compared to the non-tinted sunscreen, and 90% of the subjects reported that their melasma had less worsened compared to previous summers. Finally, both sunscreens showed very good tolerance.

Conclusion: This study showed that even in summer, the use of a sunscreen with very high UVB and UVA photoprotection reduces melasma severity, and more interestingly, the addition of visible light protection via an adapted tinted in sunscreen significantly reduces the melasma relapse.

Consumer co-creation to enhance solar protection education! A Mix and Match concept.

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Introduction & objectives: The deleterious effects of excessive sun exposure are now well established, and because of our lifestyle, photoprotection has become a major public health issue. Solar prevention campaigns reach more and more consumers, but their effectiveness remains imperfect in the countries of the northern hemisphere (SAFE* survey). In order to meet specific photoprotection challenges, we developed 2 customizable sets of products that consumers could use depending on their daily needs, activities and expectations, with the objective to introduce a right daily routine protection. These sets are composed of a very high sunscreen SPF50$^+$ and a specific daily cream (according to skin type) used as stand-alone or mixed together depending on consumer needs.
This work was to investigate consumers understanding and usage of these sets, as well as ensuring the photoprotective efficacy of mixes. We present here evaluations carried out "in house" by a coconstruction study regrouping photobiology and qualitative tests and results confirmation by independent laboratories.
Materials & Methods: A sunscreen SPF50$^+$ and a dermo-cosmetic facial cream dedicated to specific skin types (either rosacea [DC1] or normal skin [DC2]) are associated in those sets (Mix1=SPF50$^+$+DC1; Mix2=SPF50$^+$+DC2). Preliminary studies identified the quantity of each product to be mixed (2gr of SPF50$^+$/ 1gr of DC1 or DC2) to match a SPF30 final product. A combined study including photobiology efficacy and qualitative test (face to face interviews methodology) was conducted, in our laboratory, to investigate/improve (1) the understanding of the information highlighted on pack with targeted consumers and define strengths and weaknesses of sets, (2) the easiness of products handling to mix them (3) the sun protection factor (SPF) in real conditions of mixing by consumers. The finalized instructions were given to independent laboratories to confirm SPF (ISO24444 standard) and UVAPF/critical wavelength (ISO24443 standard) of such mixes in both laboratory (with precision scale weighing) or real conditions preparation by volunteers.
Results: The combined study helped us to better adjust the concept to consumers expectations and to highlight the communications elements to fit with their goal "to easily explain set's purpose". Joint analysis of the interviews and SPF results allowed us to finalize mixing instructions by making appropriate adjustments on the operating mode. Each mix condition tested (made in laboratory, by consumers or volunteers) has demonstrated its efficacy to protect from UV (*in vivo* $SPF_{Mix1\ or\ Mix2}$>30; UVA-$PF_{in\ vitro}$ > 1/3 SPF;CW>370nm).
Conclusion: A co-creation process has been set up to meet the aim of bringing a set of 2 products to every life's moment with the right efficacy (a cream for daily routine, a sunscreen SPF50+ for intense sun exposure/beach, mixed matching a sunscreen SPF30 to enhance daily sun protection when needed). It included listening to consumer experiences and acting accordingly while maintaining an evaluation process compliant with ISO standards and sun protection recommendations.

*SAFE Survey: Sun exposure and photoprotection: Parents and grandparents habits, knowledge and attitudes towards children - JEADV Clin Pract. 2022;1–6.

Photoprotective potential of topolin and its mesylate in human skin cells

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Cytokinins are purine based phytohormones that control many cellular processes in plants especially those related to senescence. The first recognized was kinetin (KIN; N^6-furfurylaminopurine) that is currently used in several skincare preparations for its antioxidant, cell proliferation-promoting and antiageing properties (1). A structurally close 6-(4-hydroxybenzylamino)purine, *para*-topolin (*p*T) described as inactive cytokinin, has been recently reported to improve skin quality, coarse wrinkles, skin roughness and non-inflammatory acne lesions (2). As *p*T is poorly soluble in water, its mesylate salt (*p*TM) that is several orders of magnitude more soluble in water was prepared. In this study we assessed basic characteristics (photostability, phototoxicity) and photoprotection of *p*T and *p*TM for potential dermatological application.

Normal human dermal fibroblasts (NHDF) were pre-treated with the compounds and exposed to UVA radiation. In several time-points, cells and media were collected and their effect on cell viability, parameters of oxidative stress and expression of selected proteins were evaluated in comparison with KIN. The effect of mesylation of parent *p*T molecule on biological activity in skin cells was evaluated as well. Results will be discussed at the poster presentation.

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Evaluation of the biological effect in DNA damage repair of a high broad spectrum with nicotinamide and panthenol, in actinic keratoses and subclinic actinic keratoses

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Background: Nicotinamide is the precursor of NAD (nicotinamide adenine dinucleotide), an essential coenzyme in the production of ATP (adenosine triphosphate), the main source of cellular energy. Previous studies in mice reveal that oral consumption or topical application of nicotinamide prevents immunosuppression and reduces the number of tumors induced by UV radiation [1]. In humans, topical application of 5% nicotinamide prevents immunosuppression caused by solar UV radiation, but not burns [2]. Furthermore, oral nicotinamide reduces the rate of diagnosis of new non-melanoma skin cancers and actinic keratoses in high-risk patients [3]. It has been suggested that one of the mechanisms by which nicotinamide may protect against photodamage is by increasing ATP production which enhances DNA repair [4]. Additionally, nicotinamide acts as a PARP1 inhibitor. Extensive DNA damage leads to overactivation of PARP1 which can lead to NAD depletion. Thus, cells are unable to enter apoptosis, since the process requires a large amount of energy [5].

Hypothesis: Using a high broad spectrum UVB-UVA, SPF 50+ sunscreen, containing Nicotinamide and Panthenol can help reverse chronic sun damage to the DNA of skin cells.

Objectives: To determine the biological effect of a high broad spectrum UVB-UVA sunscreen containing Nicotinamide and Panthenol, on DNA damage repair by measuring the presence of 6-4 PD by immunohistochemistry in patients' biopsies and indirectly by measuring the expression of p53, p21, PCNA and DIMTIM.

Methods and study design: Prospective unicentric study, with a single group of individuals. We included 12 patients with actinic keratosis who were between 50 and 70 years old. We selected four lesions of each patient in the scalp: 2 actinic keratoses (AK) and 2 subclinic actinic keratoses (SAK). In the screening visit we performed a biopsy of one AK and one SAK. After 8 weeks of applying a high broad spectrum UVB-UVA sunscreen containing Nicotinamide and Panthenol, twice a day. We performed a biopsy of the two remaining lesions. Afterwards, we performed a direct comparison between AK and SAK pre and post treatment for each immunohistochemistry (IHC) techniques. Among those IHC techniques that showed nuclear improvement, we evaluated the number of high positive nuclei and positive nuclei in a region of 50 nuclei for AK and SAK pre and post treatment.

Results: We observed statistically significant differences comparing p21 and DIMTIM high positive nuclei per 50 nuclei in AK and SAK lesions pre and post treatment. In addition, we also found statistically significant differences comparing p21 positive nuclei per 50 nuclei but not in the DIMTIM comparison. All the other IHC markers (p53, 64PPS and PCNA) did not show a nuclear improvement pre and post treatment.

Conclusions: A high broad spectrum UVB-UVA sunscreen containing Nicotinamide and Panthenol has shown a nuclear cell improvement in p21 and DIMTIM IHC techniques. Therefore, this sunscreen with niacinamide might help reverse chronic sun damage to the DNA of skin cells in our patients.

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Body Distribution of Skin Colour, Pigmentation and Degree of Tan in Central European Caucasian Women

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Background: Measurements of human skin colour and pigmentation were the center of interest in many studies and find usage in various fields like dermatology or cosmetics. Therefore, the knowledge on the differences in skin colour at different body sites and between seasons is well established by now. On the other hand, there is still an underrepresentation of quantitative data about the topography of skin colour and pigmentation.

Material and Methods: Our study aims to add to this lack of data by cutaneous colorimetric measurements at 18 body sites in 20 central European Caucasian women, ranging from age 20 to 60. To depict skin colour, tri-stimulus L*a*b*-values, hue, and chroma were considered. In addition, this study also introduces the degree of tan which, based on the individual typology angle, describes the difference between constitutive and facultative pigmentation. For estimation of potential changes due to solar radiation, the skin colour of each participant was measured twice, first in late winter and again in early summer.

Results: Results for late winter showed that skin colour differs across the body and that even nearby body sites are recognizable as differently coloured. Further, a certain degree of tan remained until late winter at permanent as well as intermittently exposed body sites and was actually most pronounced on the intermittently exposed shoulder instead of permanently exposed body parts. For early summer, results showed that the degree of tan up to this point developed most at the hands, arms, and instep, followed by the face.

Conclusion: We were able to show that, in addition to basic colour differences between body sites in winter, body parts also vary in their response to UV-radiation. Therefore, consideration of body site and time of the year is indispensable when analyzing or comparing skin colour and pigmentation.

Evaluation of the biological effect of a high broad-spectrum sunscreen containing Nicotinamide and Panthenol in actinic keratosis

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Introduction: Nicotinamide is a NAD precursor. Topical application prevents UV-induced immunosuppression and apoptosis and increases the production of ATP, cell cycle activity and DNA repair.

Objective: This study determined the effect of a high broad-spectrum UVB-UVA sunscreen containing Nicotinamide and Panthenol in actinic keratosis (AK) and perilesional skin (PS).

Materials & methods: 16 subjects, all male and >65 yo applied Nicotinamide sunscreen daily on AK lesions and PS for two months. Biopsy samples were obtained before and after treatment. RNA was extracted for sequencing.
Three differential expression analyses were conducted comparing gene expression before and after treatment: one with all the samples, another with AK lesion samples and the last one with PS samples. Analyses were paired for each patient. Genes with a significant differential expression (FDR p-value <0.05) before and after treatment were considered the differential expressed genes (DEG). Pathway analyses of the resultant differential expressed genes of were conducted using hiPATHia tool from Babelomics platform.

Results: Differential gene expression analysis revealed 128 significant genes in all samples. The analysis of AK lesion samples showed 1555 significant genes and 40 genes in PS samples.
Three genes were significantly expressed in the 3 comparisons; 17 genes were common in the analysis of all the samples and the AK samples. Four significant genes were common in the analysis of all samples as well as in the PS samples.
Pathway analysis showed that 30 circuit categories in AK and four in PS after treatment were down-expressed. Circuit categories relate to cell cycle, glucose homeostasis, and pathways in cancer. In AK they also relate to muscle contraction, apoptosis, sphingolipid, and p53 signalling pathways, cell communication, and proteoglycans in cancer.

Conclusion: This study shows that the tested high broad-spectrum UVB-UVA sunscreen containing Nicotinamide and Panthenol produces changes in AK skin and perilesional skin.

Key words : photoprotection / broad spectrum sunscreen / actinic keratosis / UV-induced damages / photo-damages / gene expression / cancer pathways / RNA sequencing

Does silymarin provide the photoprotective effects found in vitro under ex vivo conditions?

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Biological activities of substances are mainly evaluated on 2-D cell culture models. However this conventional approach has limitations compared to 3-D models. Human skin explants (HSE) are relatively cheap 3-D model for evaluation of complex effects of physical (UV radiation) and chemical factors (irritants, phototoxic and photoprotective substances) on cutaneous tissue. The aim of our study was to evaluate UVA photoprotective properties of silymarin (SM, a standardized polyphenol rich extract from *Silybum marianum* seeds) and its main polyphenolic constituent flavonolignan silybin (SB) on HSE and compare the results with data obtained on 2-D models (keratinocytes, fibroblasts)[1,2].

HSE were preincubated for 20 hours with SM and SB (12.05 and 24.1 mg l^{-1}; these concentrations correspond to 25 and 50 □mol l^{-1} of SB) and then exposed to the UVA dose of 40 J/cm^2. HSE were collected in several time intervals (6, 24, 48 hours) and used for evaluation of HSE viability (reduction of tetrazolium chloride), estimation of specific enzymes activity and content and imunohistochemical localization of selected enzymes.

UVA-protective properties of SM and SB on HSE will be discussed at our poster presentation. Results on 2-D and 3-D model will compared as well.

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Protection against UVA1 damage with the new MCE filter improves the anti-photoaging activity of Vitamin C

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Skin photoaging is a cumulative process where damages induced by chronic exposure to ultraviolet (UV) rays add to intrinsic aging. Repeated UV exposure results in the disorganization of dermal extracellular matrix (ECM) through induction of matrix metalloproteinases (MMPs) and decreased collagen synthesis (1). UVA1, the most penetrating UV wavelengths, are key contributors of dermal alterations during photoaging (2).

In this study, we hypothesized that UV exposure could antagonize the well-known stimulating effects of Vitamin C on collagen synthesis and that protection against UVA1 wavelengths with the new filter methoxypropylaminocyclohexenylidene ethoxyethylcyanoacetate (MCE) (3) could improve the anti-aging efficacy of Vitamin C under UV exposure.

Normal human fibroblasts were exposed daily to UVA for three days, under PMMA plates coated with Placebo or formulas containing MCE filter (1 to 3%). Vitamin C (1 to 100 µM) was added in the culture medium after each exposure. Pro-collagen I secretion was quantified in supernatants by ELISA (Abcam). Full-thickness reconstructed skin was exposed to UVA, with or without formulas containing MCE filter on PMMA plates. Vitamin C was added in the culture medium before and after exposure. 48h after UVA exposure, morphology analysis was performed on HES-stained sections, pro-collagen I and MMP1 secretion was analyzed by ELISA (Takara and Abcam).

Vitamin C induced a dose-dependent secretion of pro-collagen I in fibroblasts, whereas repeated UVA exposure decreased the secretion of pro-collagen I in non-treated fibroblasts. Moreover, repeated UVA exposure decreased the amount of pro-collagen I secreted by fibroblasts treated with Vitamin C, with a near complete inhibition of the stimulating effect of Vitamin C for doses higher than 25 J/cm². Addition of formulas containing 2% UVA1 filter MCE during UVA exposure increased by 50% the amount of procollagen I secreted by fibroblasts treated with 10 µM Vitamin C.

In full-thickness skin, vitamin C treatment increased procollagen I secretion and dermal fibroblast number. Exposure to UVA triggered dermal fibroblast disappearance, associated with pro-collagen I reduction and increased MMP1 secretion. Exposure to UVA in the presence of both MCE filter and vitamin C significantly improved the protection against UVA-induced fibroblast alterations, MMP1 secretion and reduced procollagen I secretion.

In this study, we showed for the first time that the association of the new UVA1 filter MCE with vitamin C significantly improved the anti-aging efficacy of Vitamin C in a context of UVA exposure, through increased synthesis of pro-collagen I and decreased secretion of MMP1. These results highlight the interest of associating strong UVA1 photoprotection to efficient compounds stimulating ECM synthesis to provide protection against the deleterious impacts of repeated UV exposure in the dermis.

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Physical and chemical photobiology

P69	**Cornelia Böhm**	Structural Determinants of Bacteriophytochrome Photocycle
P70	**Elisabetta de Diana**	Photostability of a therapeutic monoclonal antibody, NivolumabOpdivoÂ®, in its formulation and sterile saline or glucose solutions for parenteral administration.
P71	**Paolo Di Mascio**	Singlet molecular oxygen in biological systems: Mechanistic studies using 18O-labeled oxygen, mass spectrometry, and light emission measurements
P72	**Thierry Douki**	Mathematical approaches to the evaluation of non-additive effects of different UV ranges in photobiological processes
P73	**Linda Eijsink**	Shining Light on the Mutual Interaction of Photo-responsive Labels and Biomolecules
P74	**Massimo Olivucci**	From photon to neuron: the molecular mechanism of the primary event in vision
P75	**Viktoria Pevna**	Investigation of hypericin fluorescence in protein coating of nanoporous silica particles
P76	**Andrés Thomas**	Photoinduced damage to proteins and their components by an endogenous sensitizer of biomedical interest

Structural Determinants of Bacteriophytochrome Photocycle

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Phytochromes are photoreceptors sensitive to red and far-red light that can be found in a wide variety of organisms, including plants, fungi, and bacteria. The photosensory module of bacteriophytochromes, defined by a PAS-GAF-PHY domain architecture, has been found to be linked to numerous different output modules (OPM). One well-studied representative is *Dr*BphP from *Deinococcus radiodurans* bacteria featuring a histidine kinase OPM. Interestingly, output modularity can be up- or downregulated by illumination, even among different histidine kinases[1]. In "prototypical" bacteriophytochromes, the red light illuminated (P_{fr}) conformation acts as its photoactivated state, whereas the same conformation is a resting state in "bathy" phytochromes[2]. In this study, we aim to identify the main contributors to bathy vs. prototypical bacteriophytochrome behaviour by way of chimeric constructs between the prototypical *Dr*BphP and representative bathy phytochromes. Several main players are assumed to play a role in defining spectral properties of bacteriophytochromes including the PHY-tongue, a hairpin extension involved in chromophore stabilisation, as well as the N-terminal segment (NTS). Preliminary results confirm that the *Dr*BphP photosensory module controls OPM functionality regardless of PHY-tongue origin. Further experiments will also consider the NTS, and a potential interplay between NTS and PHYtongue. Better understanding of the factors governing spectral and functional qualities provides an essential basis for photoreceptor engineering and generation of phytochrome-based optogenetic actuators.

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Photostability of a therapeutic monoclonal antibody, Nivolumab-Opdivo®, in its formulation and sterile saline or glucose solutions for parenteral administration.

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Monoclonal antibodies (mAbs) are complex protein molecules, and their structural integrity impacts on their biological and pharmacological activity, i.e., against cancer and pathogens, which stability under storage conditions and during their management represent a fundamental parameter. We investigated the impact of some stress factors, i.e., shaking/vibrations, temperature, dilution and light exposure, on the formulated anticancer monoclonal antibody Nivolumab (Opdivo®) with or without dilution (saline or glucose solutions). The indoor and outdoor light exposure is such a critical exogenous stressing factor because a drug product could be photo-exposed during the transport or within the hospital and even during the time of administration to the patient. The mAb stability (*e.g.,* aggregation and dilution effects) was carried out by biochemical and biophysical analyses. The biochemical and biophysical results showed that Nivolumab is quite stable under vigorous shaking and moderate temperature (37°C) for 45 days. However, it undergoes structural change, mostly aggregation and AA oxidation, upon exposure to artificial sunlight. The dilution media used for administration to the patients, in particular the sterile glucose solution containing degradation glucose products, should be considered in terms of photostability of the drug product.

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Singlet molecular oxygen in biological systems Mechanistic studies using ^{18}O-labeled oxygen, mass spectrometry, and light emission measurements

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Evidence has been accumulated during the last three decades on the strong implication of several oxidants including hydroxyl radical, one-electron oxidants and singlet molecular oxygen [$^1O_2$] in the generation of hydroperoxides from several nucleobases, amino acids and unsaturated lipid components. We focus on reaction mechanisms and the characterization of the products formed from the reaction of specific oxidants, $^1O_2$ and peroxides, with specific biomolecules and the development of robust and sensitive methodologies to detect these products in biological samples (Di Mascio et al. Chem Rev. 2019). Participation in an international network resulted in the first demonstration of $^1O_2$ and peroxide involvement in mammal physiology (Stanley et al. Nature 2019). In irradiated biological system, UVA photons are poorly absorbed by the DNA, being more relevantly absorbed by other cellular chromophores. In this sense, UVA relies on the generation of hydroperoxides and photoexcited species, such as $^1O_2$. Using subcellular fractionation, mass-spectrometry-based proteomics, machine learning algorithms, immunofluorescence, and functional assays, we mapped the subcellular reorganization of the proteome of human keratinocytes in response to UVA light. Mitochondria were identified as one of the main targets of UVA-induced stress. We also showed that UVA modulates the secretory phenotype of these cells to the extent of inducing paracrine oxidative stress and immune system activation in pre-malignant keratinocytes (Valerio et al. Sci Rep. 2021; Valerio et al. iScience 2022). Methionine is one of the main targets for biological oxidants. Its reaction with the majority of oxidants generates only methionine sulfoxide. However, when N-terminal methionine reacts with hypohalous acids (HOCl and HOBr) or $^1O_2$, it can also generate a cyclic product called dehydromethionine (DHM). Further studies will be undertaken to evaluate the role of $^1O_2$ in neutrophils and eosinophils under activation (Nascimento et al. Free Radic. Biol. Med. 2022). Despite generating DHM, reaction of $^1O_2$ with tryptophan and tyrosine (either free or inserted in proteins) generates hydroperoxides. (Jayme et al. Photochem. Photobiol. 2022). The approach used to unequivocally demonstrate the generation of $^1O_2$ in these reactions is the use of ^{18}O-labeled hydroperoxide / triplet dioxygen ($^{18}[^3O_2]$), the detection of labeled compounds by HPLC coupled to mass spectrometry (HPLC-MS^n) and the direct spectroscopic detection and characterization of $^1O_2$ light emission. The combined use of the thermolysis of a water-soluble naphthalene endoperoxide as a generator of ^{18}O labeled $^1O_2$ allowed the study of $^1O_2$ reactivity toward biomolecules. Photoemission properties and chemical trapping clearly demonstrate that the production of hydroperoxides and excited carbonyls (ACS Chem. Biol. 2023) generates $^{18}[^1O_2]$, and points to the involvement of $^1O_2$ in physiological and pathophysiological mechanism.

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Mathematical approaches to the evaluation of non-additive effects of different UV ranges in photobiological processes

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Photobiological effects are known to greatly depend on the wavelength of the incident photons that define the nature of the activated chromophores. This is reflected in action spectra that visualize the efficacy of photo-induced processes at individual wavelengths. However, a growing number of experimental data show that considering the effect of complex light sources as a sum of the effects of monochromatic exposures can be misleading. Indeed, the combined exposure to several wavelength ranges may modulate photobiological responses or even induce specific processes. These observations are similar to a well-known topic in chemical toxicology: the non-additivity of effects in mixtures where either antagonism or synergy are often observed. These "cocktail effects of radiations" are not easy to study experimentally and careful data treatment, based on abundant and high quality experimental information, is necessary. Using formation of pyrimidine dimers in isolated DNA as model photoreactions, we first investigated if a data analysis tool first developed for studying non-additivity in mixtures of drugs, the combination index (CI), could be applied to photobiological processes. We showed that the values of CI nicely reflect the additive formation of cyclobutane pyrimidine dimers between UVB and UVA. The conversion of UVB-induced (6-4) photoproducts into their Dewar valence isomers by UVA was also shown by an inhibitory effect of the combination of the two wavelengths ranges for the former damage and synergy for the latter. CI appears thus as an efficient tool to establish non-additivity in complex UV sources. Yet it does not quantitatively predict the impact of combination of radiation. For this purpose, we are currently developing a first approach involving experiment plans that will provide a polynomial equation describing the formation of the photoproducts under different exposure scenarii. Another more complex strategy relies on artificial intelligence. The ultimate goal is to provide multidimensional action spectra taking interaction between wavelengths in account.

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Shining Light on the Mutual Interaction of Photo-responsive Labels and Biomolecules

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Light is an excellent external stimulus in chemistry and biology, as it provides high spatial and temporal resolution. The implementation and use of photocages in peptides in proteins was recently reviewed.[1] Applications include light-controlled cell apoptosis[2] or live cell patterning.[3] The introduction of photoresponsive modification into peptides and proteins can be achieved either by incorporating them synthetically as unnatural amino acids (UAAs), by reacting UAAs in bioorthogonal transformations, or by sidechain-selective labelling.[4,5]

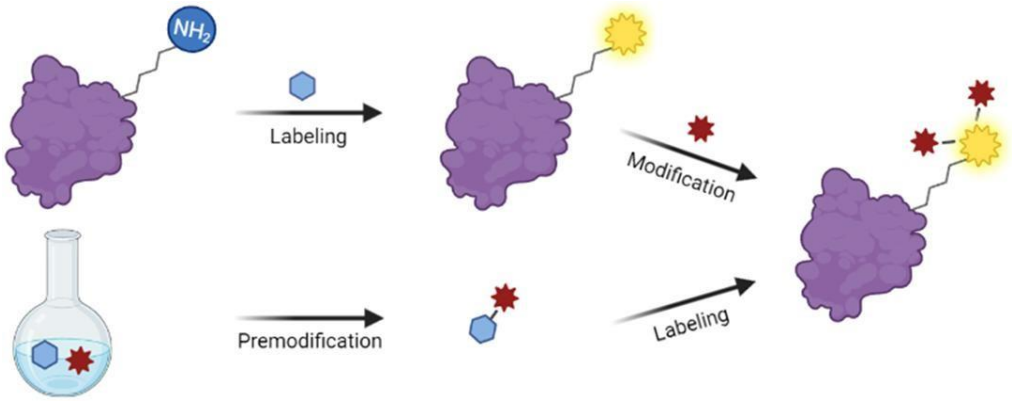

Figure 1: Post- or premodification of a fluorescent tag allows for further functionalisation of a native protein.

Irradiation with visible light is generally considered non-invasive for biological systems. However, the introduced light-sensitive unit potentially affects the targeted biomolecule in its 3D-shape and function. Vice versa, the change in environment upon ligation to a biomolecule can impact the photophysical and photochemical properties of the photo-responsive entity. Thus, identifying suitable small organic handles such as fluorescent labels or photocages is of key importance. The wavelength of choice introduces further complications: small organic molecules are generally responsive to UV radiation, whereas biological applications require irradiation with visible light. Introduction of functional groups allows tuning of the properties accordingly, though adding additional requirements to the molecular design.

Here, we present the molecular functionalisation of small biomolecules, as well as the photochemical studies of their formation and/or disappearance and their properties. Using in-situ irradiation with a combination of UV/Vis, fluorescence, NMR, and mass spectroscopy, we are able to shine light on the interaction of organic chromophores and biomolecules and derive structure-property relationships.

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From photon to neuron: the molecular mechanism of the primary event in vision

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The activation of rhodopsin, the light-sensitive G-protein coupled receptor responsible for dim-light vision in vertebrates, is driven by an ultrafast excited state double-bond isomerization with a quantum efficiency ($\Phi_{cis\text{-}trans}$) of almost 70%. The origin of such a high light sensitivity, ultimately allowing the human eye to detect even single photons, is not understood. A key unanswered question is whether and how the level of

synchronization between different receptor vibrational modes controls the $\Phi_{cis\text{-}trans}$ value. Here, we employ hundreds of quantum-classical trajectories to show that, 15 femtoseconds after photon absorption the excited state population of rhodopsin splits into subpopulations reacting with different velocities and leading to distinct contributions to $\Phi_{cis\text{-}trans}$. We find that each subpopulation and $\Phi_{cis\text{-}trans}$ contribution, is associated with a different phase relationship between specific critical vibrational modes. We also show that the population splitting is modulated by the protein electrostatics, thus linking amino acid sequence variations to $\Phi_{cis\text{-}trans}$ modulation.

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Investigation of hypericin fluorescence in protein coating of nanoporous silica particles

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In this work, we present preliminary results of the interaction of photodynamically active hydrophobic molecule – hypericin with nanoporous silica and cells. The architecture of nanoporous silica, has been exploited in the last decades for numerous applications, and allows them to bind and fill with various molecules - both hydrophylic and hydrophobic. Silica nanoporous particles have been reported to dispose crucial advantages like their greater surface area, big pore volume, mechanical and chemical stability, biocompatibility very low toxicity and upgradeable pore sizes. The porous character allows to adsorb hydrophobic and hydrophilic fluorophores. Our aim was to investigate the capability of these particles to adsorb proteins and hypericin. The ability to release hypericin from particles was studied by fluorescence spectroscopy and confocal fluorescence microscopy. The observation brought findings, that protein corona enhances hypericin redistribution from nanoporous silica particles towards cancer cells. Singlet oxygen was produced thanks to monomeric hypericin formed in protein corona. It suggests that this complex silica-hypericin possesses promising properties in photodynamic therapy (PDT). PDT efficacy of silica-hypericin complex was comparable to hypericin-PDT without silica particles.

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Photoinduced damage to proteins and their components by an endogenous sensitizer of biomedical interest

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Solar radiation causes modifications to different biomolecules and is involved in the generation of human skin cancer. Most of UV solar radiation that reaches the Earth's surface corresponds to UV-A radiation, which can cause damage mainly through photosensitized reactions. Photosensitization consists of the chemical alteration of a compound as a result of the initial absorption of radiation by another chemical species called photosensitzer. At tissue level the photosensitization processes can lead to the generation of neoplasic processes, and the photochemical production of reactive oxygen species (ROS), with the concomitant generation of oxidative stress. These processes are particularly important in individuals with skin diseases that cause alteration in pigmentation, the most protective mechanism against radiation.

Due to their relatively high abundance, their ability to bind chromophores, and the reactivity of particular amino acid residues, proteins are one of the preferential targets of the photosensitized damaging effects of UV radiation on biological systems. The oxidation of these biomolecules mediated by radiation may occur *via* two different pathway: i) direct absorption of radiation by the amino acid residues, ii) absorption of UV-A radiation and/or visible light *via* endogenous or exogenous photosensitizers, leading to the production of excited species which react with the biomolecule by electron transfer or hydrogen abstraction (Type I mechanism) or energy transfer to molecular oxygen to produce singlet oxygen ($^1O_2$, Type II mechanism).[1]

Pterins, a family of heterocyclic compounds, are present in biological systems in multiple forms and play different roles ranging from pigments to enzymatic cofactors for numerous redox and onecarbon transfer reactions. Under pathological conditions, such as vitiligo, oxidized pterins accumulate in the depigmented patches on the skin of patients suffering from this pathology. In aqueous solutions, these molecules are photochemically reactive and, under UV-A excitation, they can fluoresce, produce organic radical and ROS, undergo photooxidation and, photosensitize chemical modification of different biomolecules, such as DNA, proteins and lipids.[2]

In the context of our investigations related to the photosensitizing properties of pterins, this work will show the different modifications that proteins undergo, such as, oligomerization, oxidation, fragmentation and generation of protein-photosensitizer adducts, when these biomolecules are exposed to UV-A radiation in the presence of different pterin derivatives. The reaction mechanisms responsible for these processes, as well as their biological importance, will be analyzed.

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Plants, photosynthetic organisms and environment

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Degradation of PLA and PET particles exposed to UV light and toxicological effects on human intestinal cell models

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Plastics are synthetic polymers that are widely used in a large range of industrial sectors for their attractive properties and their low cost. Because plastics are highly resistant to degradation, they accumulate and pollute the oceans and soils. It is acknowledged that in the environment, plastics are subjected to physical, chemical, and biological stresses such as photodegradation, mechanical stress or biodegradation, which are responsible for plastic fragmentation and the impairment of physicochemical features. As it ages, plastic degrades into smaller fragments called micro and nanoparticles (MNPs). Aged plastic particles have been reported to show a higher specific surface area and a higher adsorption rate for pollutants than pristine particles. Hence, they are more likely to adsorb at their surface persistent organic pollutants (dioxins, pesticides), metal ions, additives or pathogens present in the environment. We are exposed daily to plastic fragments mostly via ingestion, through the consumption of fishery products, tap and bottled water. Studies on biological effects of plastic nanoparticles are still insufficient, especially on their intestinal toxicity.

In this context, our first objective was to evaluate, in realistic environmental conditions, the degradation of two different plastic particles exposed to UV light: commercial particles of polylactic acid (PLA), which is a biosourced and biodegradable polymer and representative secondary particles of polyethylene terephthalate (PET), which is a fossil-based and non biodegradable polymer derived from plastic water bottles. Our second objective was to assess biological effects of photodegraded PET and PLA particles on human intestinal cell models. These particles were exposed to UV light in a Q-SUN test chamber at 1.44 W/m2 to simulate in accelerated fashion the degradation caused by solar radiation in the environment. The biological effects of PET and PLA were evaluated on human intestinal epithelial cell lines representative of healthy individuals and of people suffering from inflammatory bowel disease. To this end, co-cultures of wild-type Caco-2 cells or Caco2-LVNod2^{1007fs} cells, which is a mutation frequently observed in Crohn's disease patients, and HT29-MTX were used. These cells were exposed to 120, 200 and 450 nm PLA particles or to 200 nm PET particles. Particles were characterized by Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and their degradation after artificial weathering was investigated via liquid chromatography coupled to mass spectrometry (HPLC-MS/MS). Cytotoxicity, DNA damage, and intracellular ROS levels were assessed after 24 h of exposure to realistic concentration of pristine or irradiated nanoparticles.

The results of the study show significant release of photoproducts corresponding to smaller oligomers of lactic acid in the suspension after UV treatment. These results suggest that exposure to UV light induces PLA hydrolysis. However, the results do not show any toxicity of PET and PLA nanoparticles on Caco-2 and HT29-MTX cell lines. These results are consistent with the literature, suggesting that the toxicity of MNPs may rather lay in their role of pollutant carrier than in intrinsic hazard, but further work is needed to confirm this hypothesis.

This work received funding by the Agence Nationale de la Recherche (ANR), under the grant ANR-21CE34-0028 (PLASTOX) and from the Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES) under the grant EST-21-077 (EXaMINA).

Optimizing Chloroplast Expansion Microscopy

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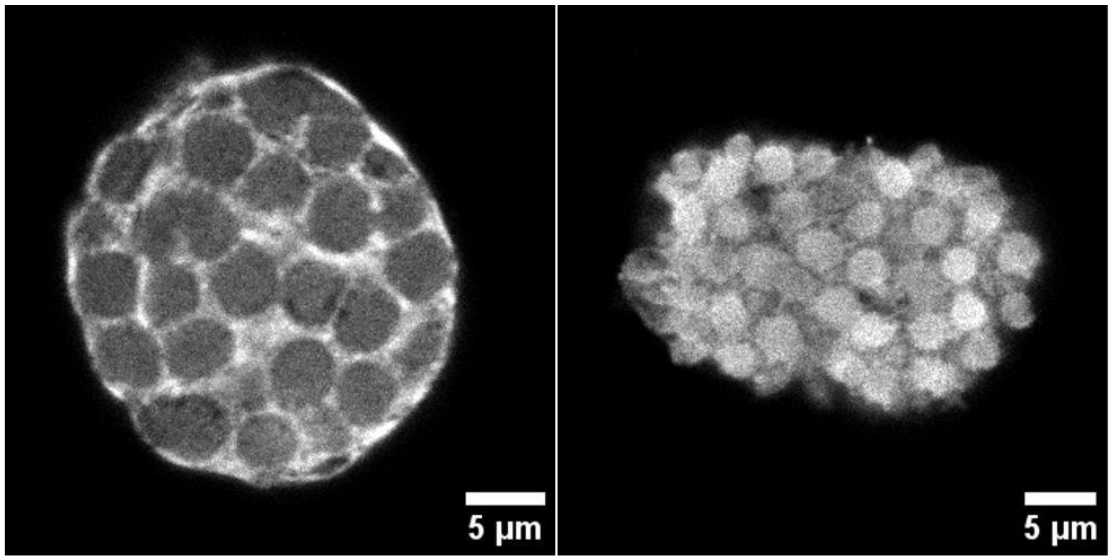
In plants, the light-driven reactions take place on the thylakoid membrane of chloroplasts. This membrane consists of the grana stacks and the stroma lamellae that connect these grana. The thylakoid is a highly dynamic structure, able to change grana dimensions depending on light conditions. Although much research has been done to elucidate the thylakoid ultrastructure, several elements of its organization and reorganization remain unclear, as current state-of-the-art microscopy techniques are unable to fully elucidate the 3D thylakoid ultrastructure.

Expansion microscopy is an exciting new technique, able to elucidate the 3D ultrastructure of biological samples by expanding these samples. This technique could be a vital tool to study the thylakoid membrane ultrastructure. However, expansion microscopy protocols need to be optimized for every sample. Here, we present the current state of chloroplast expansion microscopy. We are able to image expanded isolated intact chloroplasts and intact thylakoid membranes from *Spinacia oleracea*. Furthermore, protoplasts of *Arabidopsis thaliana* could be expanded using a similar protocol, showing chloroplasts in a near native state.



Expanded chloroplast with intact envelope Expanded chloroplast without intact envelope

Characterization of photosynthesis and photoprotection mechanisms in marine cyanobacteria

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While harvesting the sun's energy enables cyanobacteria to carry out photosynthesis, too much of this energy can be toxic. It can lead to the generation of reactive oxygen species (ROS), which can provoke the cell death. Thus, photosynthetic organisms evolved protective, nonphotochemical quenching (NPQ) mechanisms to safely dissipate excess light energy. Cyanobacteria uniquely contain a soluble carotenoprotein, the Orange Carotenoid Protein (OCP), binding to a single carotenoid molecule. Upon activation by blue-green light, OCP converts from a stable orange form, OCP^O, to a light-activated red form, OCP^R. OCP^R directly participates in a photoprotective mechanism by binding to the phycobilisome (PBS) at the allophycocyanin core. It dissipates safely the excess of energy, captured by the pigments within the PBS, as heat, thereby preventing ROS formation. To date, OCP proteins have been well studied in freshwater cyanobacterial models, however very little is known about marine OCPs. Our bioinformatics analysis revealed that OCP from marine *Synechococcus* is relatively poorly conserved. Only 65% sequence identity to the OCP1 of freshwater strains (typically 85-90% identical); therefore, they are the most common divergent OCP. We hypothesized that the differences in the primary structure will be reflected in the photoconversion kinetics. In our current work, we are biochemically characterizing OCP from three different marine *Synechococcus* strains reflecting different ecological environments in the ocean. Moreover, we are determining the *ocp* gene expression under different conditions such as high-light or darkness. Finally, we are identifying and quantifying the pigment content, with specially interest on carotenoids, under different conditions. Altogether, we will shed light on the regulation of this important physiological process, photosynthesis and photoprotection, in those ecologically relevant species.

Regulation of phototropin promoters from *Arabidopsis thaliana*

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Phototropins are plant photoreceptors, which perceive blue and UV light and help plants to sense the surrounding environment, especially to optimize the efficiency of photosynthesis. In *Arabidopsis thaliana* two genes: *PHOT1* and *PHOT2* encode phototropins with overlapping physiological functions [1].

Light regulation of phototropin transcript levels is maintained during the whole period of growth of *Arabidopsis*. It is observed that *the PHOT1* transcript is downregulated whereas *the PHOT2* transcript is upregulated by light. These light-dependent changes may be regulated at the transcriptional level with alteration of promoter activity by Transcription Factors (TF), yet, no TFs have been characterized. Based on bioinformatics analysis and Yeast One Hybrid assay several light-regulated motifs and transcription factors binding sites in phototropin promoter regions have been identified [2].

To confirm transcriptional regulation of phototropins' expression we investigated the activity of their promoters using the GUS (β-glucuronidase) histochemical staining assay on transgenic lines under the control of phototropin1 (-3000nt - 1nt) and phototropin2 (-661nt - 1nt) putative promoter sequences in plants of different developmental stages and grown in different light conditions. Additionally, we analyzed transcript abundance of phototropin genes in wild-type plants grown in the same conditions. Expression levels of both phototropins corresponded to the activities of their promoters in terms of plant organs and developmental stages. Phototropin expression was also investigated in T-DNA mutant lines of identified TF genes (A-PTF-1, TCP2, ANAC 102, ARF19, EIL2, and ATHB-12) in two light regimes: darkness and after irradiation with strong white light, to induce responses of both phototropins (*PHOT1 and PHOT2*). We did not observe any pronounced effect of *PHOT1* and *PHOT2* expression in *Arabidopsis* transcription factor mutants. In the presence of exogenous auxins, *PHOT1* and *PHOT2* transcript levels are regulated in ARF7 dependent manner. In the next approach, to confirm the interaction between *PHOT2* promoter and TF we will check their activities in the presence of TFs with a GFP tag *in planta* using tobacco transient expression and EMSA assay.

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The phenomenon of light-responsive cellulose synthesis in bacteria

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Bacterial cellulose (BC) produced by many species of bacteria such as *Komagataeibacter*, has many niche applications in medicine, sensors, packaging, tissue scaffolds, and composites etc[1]. As a growth associated product, BC synthesis process is influenced by several factors such as oxygen, pH etc. but, the influence of external light is not well studied[2]. It has been hypothesized that the cellulose synthesis is a shielding mechanism of the bacteria in response to the potentially lethal ultraviolet light exposure, but it lacks strong experimental evidence[3]. On the other hand, the critical role of c-di-GMP in the activation of bacterial cellulose synthase (BCS) in bacteria have been reported. The intracellular concentration of c-di-GMP is also known to be influenced by the light in various life-forms[4]. So, the mechanisms behind the light-influenced c-di-GMP concentration in various bacteria and the activation of BCS by c-di-GMP in the BC producing bacteria provides a strong theoretical background to study the unknown effect of light on cellulose synthesis.

Here we present our recent research findings on the BC synthesis by *Komagataeibacter medellinensis* bacteria under continuous exposure of different wavelengths of light in the visible spectrum. Wavelengths of 395nm (violet), 460nm (blue), 515nm (green), and 630nm (red) were independently studied at different photon flux densities (PFD). The highest increase of 50% in the BC yield was observed with violet light at the optimal PFD of 12 µmol $m^{-2}s^{-1}$ compared to the reference (no light). Similarly, an increase in the BC yield of up to 40% was observed with blue, green, and red lights at their optimal PFDs. Interestingly, a different optimal PFD was observed for each wavelength. Additionally, we investigated the molecular response mechanism behind the bacteria-light interactions and how the exposure to light affects the porous structure of the BC. This study further contributes to the fundamental understanding of light-mediated BC synthesis and regulatory mechanism behind it. Further, this work provides a novel tool to engineer cellulose at the molecular level which can be exploited using synthetic biology tools.

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Photochemical and growth response to inclusion of algal biomass in the growth medium of lettuce (*Lactuca sativa*)

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An innovative project, ALGECO, focusing on nutrient removal from wastewater effluent using two strains of filamentous algae, *Stigeoclonium* and *Oedogonium*, is currently underway, and further use of the algal by-products is being investigated. Preliminary results from trials in the use of dried algal biomass as a slow-release fertiliser have indicated that algal inclusion in peat growth medium increased the effective quantum efficiency of PSII photochemistry (Φ_{PSII}), photochemical quenching (qP) and electron transport rate (ETR), despite no difference in Fv/Fm or Fv'/Fm' in Crispi lettuce (*Lactuca sativa* cv. Frillice). Additionally, algal inclusion increased leaf biomass production and chlorophyll content of the leaves. Combined, these results indicate a positive effect of algal inclusion on nutrient uptake and thereby plant growth. Both photochemical responses and nutrient uptake are affected by the light regime during growth, indicating the potential enhancement of plant growth for production through combining algal inclusion in the growth medium and optimal light conditions. Future experiments will further investigate the effect of algal inclusion on plant photochemistry and growth, as well as the effect of light on nutrient uptake from algae as a slow-release fertiliser. The project, preliminary results and future planned experiments will be presented.

Nitrogen-Doped Indium Quantum Dots for plant-plant nutrient transfer.

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Nowadays 4.4% of the planet is cultivated which leads to a high worldwide demand for Nfertilizers. This situation has caused an environmental problem due to the contamination of water bodies by excess of nitrates. And it tends to be exacerbated in recent years by agriculture intensification. That is why in 2020 United Nations set an ambition to halve nitrogen waste from all sources by 2030.

To accomplish this goal, developing new sustainability agriculture techniques that contribute to reduce fertilization are needed. One could be focus on plant-plant nutrient transfer. [1] Moreover, field experiments have shown that nutrients such as nitrogen (N), phosphorous (P), water and carbon (C) [2] can be transferred among plants. However, up to date N transfer between plants has been measured using the heavy stable isotope of nitrogen (^{15}N) as tracers. [3] This technique entails several drawbacks, mainly; it only allows tracking one plant at a time and damages the plant by collecting leaf samples. This work overcomes these limitations proposing the use of an optical sensor for live tracking nutrient interactions inside plant communities with scarce water and nutrients availability.

To achieve that it has been synthesized Indium Quantum Dots (QDs) with different nitrogenbased coatings as trackers for nitrogen exchange between plants. We also show, through manipulation of this InP/ZnS QD surface, how coating charge affects nanoparticle uptake and translocation within plants.
Finally, a complete QD characterization is presented together with fluorescence experiments that measure the final QD yield in soil movement studies. Also, confocal fluorescence microscopy has been used to track the QDs within the plant.

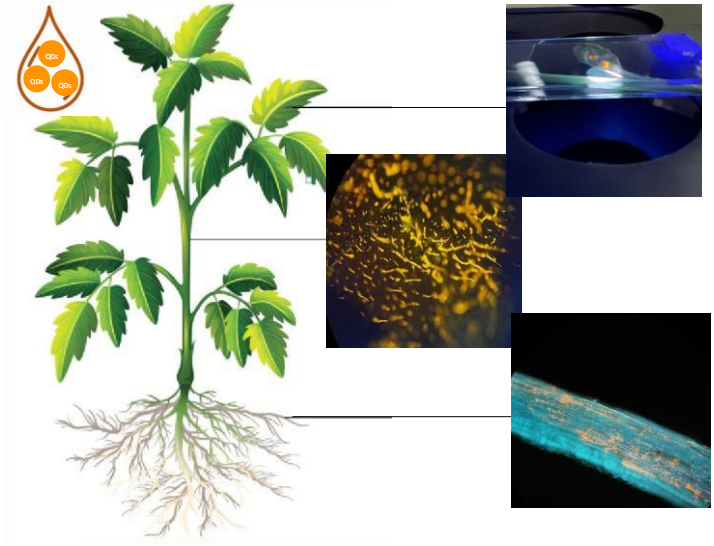

Figure. Fluorescence images of a QD internalization inside a plant.

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Ultra-sensitive simultaneous quantification of carnosine adducts by an on-line liquid chromatography-electrospray tandem mass spectrometry assay

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It is well known that exogenous and endogenous agents can modify cellular DNA. Reactive oxygen species can be produced by endogenous sources such as cell aerobic metabolism and inflammation, or by exposure to a variety of chemical and physical agents as UV light (Di Mascio et al 2019). Modification of cellular DNA upon exposure to reactive oxygen and nitrogen species is the likely initial event involved in the induction of the mutagenic and lethal effects of various oxidative stress agents. DNA lesions include strand breaks, cross-linkage, abasic sites, and modified bases. The most used marker of oxidatively generated DNA damage is 8-oxo-7,8-dihydroguanine (8-oxoGua) or its deoxyribonucleoside (8-oxodGuo). Several analytical methods have been developed to quantify oxidatively modified bases and now, wellestablished methods are available to measure 8-oxodGuo levels in DNA both *in vitro* and *in vivo*. Exocyclic DNA adducts have been also used for the study of oxidative stress-related diseases such as inflammatory, metal storage and neurodegenerative disorders, as well as the determination of cancer etiology and cancer risk. Some of these lesions can result from the reaction of DNA with lipid oxidation products, such as malonaldehyde, 4-hydroxy-2-nonenal (HNE), 4-oxo-(2*E*)-nonenal 2,4-decadienal (DDE), 4,5-epoxy-(2*E*)-decenal (EDE), hexenal, acrolein, and crotonaldehyde (Medeiros, 2009). 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, also, the ability to react with singlet oxygen, and other reactive oxygen species. It was, recently, shown that carnosine prevents proteomic alterations in the skin of hairless mice exposed to UV-A radiations. These alterations could result from lipid oxidation products generated by UV-A as HNE and acrolein (Radrezza et al 2021). It was also shown that carnosine was able to offer the protection against early and delayed UVA damages at the dermal level (Aiello et al 2023). To support the hypothesis that carnosine is important for the metabolism of unsaturated aldehydes, this work shows the development of a sensitive method using on-line reverse-phase HPLC coupled with an electrospray tandem mass spectrometer (ESI+ HPLC-MS/MS) to quantify the presence of carnosine adducts with acrolein, HNE and 4-hydroxy-2-hexenal (HHE) in cells exposed to UV-A radiation.

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This work was supported by “Fundação de Amparo à Pesquisa do stado de ão Pau o (FAP P P edo oma – “ onse ho aciona de esen o imento ien co e Tecno ico (CNPq) Grants 304945/2021-8 NAP Redoxoma (PRPUSP 2011.1.9352.1.8) and John Simon Guggenheim Memorial Foundation (PDM).

Development of chlorosome-type artificial photosynthetic antenna efficiently absorbing green light

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Chlorosomes are the main antenna systems of green photosynthetic bacteria. Bacteriochlorophyll (BChl)-*c*/*d*/*e* molecules self-aggregate inside a chlorosome to enable efficiently light absorption and rapid excitation energy transfer.[1)] Chlorosomal self-aggregates have two large visible absorption bands called the Qy and Soret bands at the red and purple-to-blue regions, respectively, but cannot efficiently absorb green light where sunlight is intense.[2)] To develop a solar-energy conversion system using artificial chlorosomes, the "green gap" problem must be solved. In some natural chlorosomes, specific carotenoids with absorbance around 500 nm are situated near the BChl self-aggregates and used for the accessory pigments where photoexcited energy transfer occurs from the former to the latter.[3)] In addition, energy transfer from photoexcited naphthalene bisimides with absorbance around 540 nm to chlorosomal self-aggregates were confirmed in artificial systems.[4)] Here we report a chlorosome-type antenna model using a boron-dipyrromethene (BODIPY) with absorbance around 500 nm as an accessory pigment.

Zinc chlorophyll-*a* derivative as a BChl-*d* model was covalently linked with a BODIPY moiety at the 17-propionate residue to give conjugate **1** (Fig. 1). Synthetic conjugate **1** in THF was monomeric to show efficient intramolecular energy transfer from the photoexcited BODIPY part to the zinc chlorin. By contrast, the zinc chlorin part of conjugate **1** self-aggregated in non-polar organic solvents. Excitation energy transfer from the BODIPY moiety to the zinc chlorin self-aggregates was observed in the supramolecule. This is another example of the artificial chlorosomal model absorbing green light.

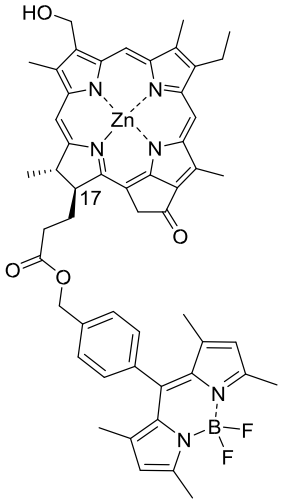

Fig. 1. Molecular structure of zinc-chlorin–BODIPY conjugate **1**.

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Characterisation of the impact of chloroplast avoidance on photosynthetic efficiency of plants in fluctuating light

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Plant's adaptation and acclimation to dynamic changes in environmental conditions depends on multiple photoprotective mechanisms. Depending on the light conditions, chloroplasts quickly change their location within the cell. In strong light, chloroplasts exhibit an avoidance response by gathering along the cell walls parallel to the direction of light. This is a response contributing to photoprotection from high light exposure [1][2]. However recent data cast doubt on its effectiveness [3]. To elucidate this apparent contradiction, we measured simultaneously chloroplast avoidance and the kinetics of chlorophyll fluorescence in plants grown in three different light conditions: fluctuating white light (550 / 50 µmol·m^{-2}·s^{-1}, 50 s each); steady white light of median irradiance (300 µmol·m^{-2}·s^{-1}); and steady white light of high irradiance (550 µmol·m^{-2}·s^{-1}). We compared eight genotypes: wild-type *A. thaliana* (Col-0), and mutants defective in chloroplast movements (*phot1*, *phot2*, *phot1 phot2*, *chup1*, *jac1*) or in photosynthetic machinery components (*npq4*, *stn7*). Changes in leaf transmittance, a proxy for chloroplast avoidance/accumulation, had larger amplitude in plants cultivated in fluctuating compared to steady irradiance. The smallest amplitude was recorded in plants from high light conditions, except for the *npq4, phot1phot2,* and *jac1* genotypes. Dualex estimates of adaxial epidermal flavonols detected significant differences, with twice the UV-A attenuation in plants grown in steady high light compared to fluctuating light. The epidermal anthocyanins were similar across all light conditions. Total carbon gain over one cycle in the fluctuating treatment was 10 to 25% lower for Col-0, *phot1, phot2, stn7,* and *npq4*, but 10 to 18% higher for *jac1, chup1,* and *phot1phot2* as compared to the steady median light. The effectiveness of chloroplast avoidance as protection from photoinhibition of photosystem II (PS_{II}) was assessed by recording the maximum quantum yield of photosystem II (F_v/F_m). To eliminate the PS_{II} repair process leaf discs were incubated with lincomycin (an inhibitor of chloroplast-encoded protein synthesis) or control solution. The results indicate a faster decline in F_v/F_m for *phot2* and *phot1phot2* mutants, which are defective in chloroplast avoidance, than in the wild-type Col-0. However, in the *chup1* mutant, defective in all chloroplast movements, the difference was smaller and is likely due to the constitutively basal positioning of chloroplasts, as visible through confocal microscopy. ImagingPAM analysis of Fv/Fm suggests that chloroplast movements and positioning are involved in photoprotection. However, this seems to be more relevant in plants grown in fluctuating light conditions, as they exhibit the biggest amplitudes of chloroplast avoidance. Gas exchange data imply that chloroplast movements in fluctuating light consisting of 50 s light phases are not a sufficient mechanism for total carbon capture optimization.

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Management of postharvest gray mold using blue light and cold storage

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Gray mold caused by cryptic species of *Botrytis* can be of significant threat to postharvest fruits and berries including strawberries. Even though cold storage can extend the shelf life of strawberries, cold adaptive *Botrytis* species are able to cause losses. While fungicides play major role in managing this disease, this strategy is neither sustainable nor an environmentally friendly. The regulatory effects of light and storage temperature conditions were examined on growth and development of *Botrytis cinerea* invitro and disease development on harvested strawberries cv. Favori and Murano. At 21 °C storage temperature, none of the tested light conditions (full spectrum white, blue, or red) caused remarkable effects on conidial germination or colony growth compared with darkness. However, blue light in combination with cold storage (4 °C) significantly inhibited conidial germination and colony expansion compared with all other lighting-storage temperature conditions. The accumulation of reactive oxygen species (ROS) in fungal hypha was significantly high in blue light-cold storage treatment compared with all other combinations. The results showed the potential of blue light in minimizing postharvest losses caused by *B*. *cinerea* in combination with cold storage. However, additional trails are necessary to adapt this technology into practical applications.

De novo proteins as biogenic matrices for creating excitonically coupled dimers with charge-transfer character

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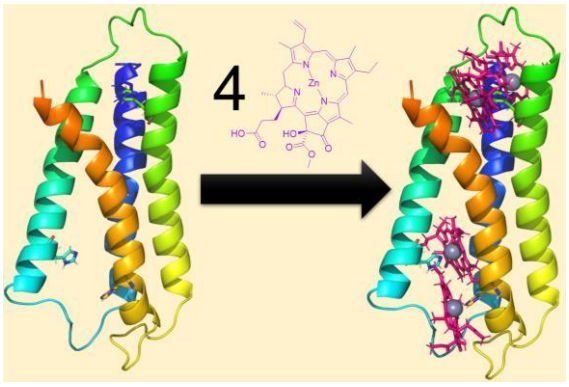
Biogenic matrices are a promising solution to minimize the use of noxious components in photonic devices.[1] A primary source of inspiration for biogenic matrices comes from the proteins of the photosynthetic machinery, where the embedded pigments efficiently capture sunlight and convert it into charge-separated states. The proteins environments play a major role in regulating these events.[2] In this sense, the *de novo* design of proteins offers biogenic matrices with potential to reproduce the role of natural ones and to be applied in the construction of devices for energy conversion. Following this approach, we utilize stable four-α-helix-bundle maquettes able to bind up to 4 chromophores (Zincpheophorbide-a, ZnPPa) by histidine binding sites. Due to the histidine positions, excitonically coupled dimers with charge transfer character could be created. The excitonic coupling was probed by circular dichroism, and fluorescence spectroscopy.[3] Finally, the charge transfer character of the dimers excited states were evaluated by Stark absorption spectroscopy.



Representative structures of ZnPPa, a de novo protein and the respective Chromophore-Protein complex.

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SPEAKERS INDEX

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