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Light signalling by vertebrate photoreceptor opsin and G-protein

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I have been interested in physiological roles and molecular mechanisms of photoreception in animals. In my earlier career, I pursued biochemical and molecular biological studies on the retinal photoreception in vertebrate visual cells. Then my interest extended to exploring the photoreception in avian pineal glands and later to the photic entrainment of circadian rhythms.

(i) Photoreception and visual transduction process: I started my scientific career in Prof. Yoshizawa lab (Kyoto University) by finding that metarhodopsin II is a physiologically active intermediate of rhodopsin (*BBA*, 1979) and that *cis-trans* isomerization at C11=C12 of retinylidene chromophore is indispensable for visual transduction (*Biochem.*, 1984). Then Yoshizawa lab purified four kinds of cone opsins for color vision, UV-Violet (SWS1), Blue (SWS2), Green (RH2), and Red (LWS) opsins, from chicken retinas (*Biochem*, 1989). cDNA cloning of the four color opsins revealed that the divergence of the color opsin genes is ancient to that of rhodopsin, a scotopic opsin (*PNAS*, 1992). Recently, Dr. Daisuke Kojima and I found that *sine oculis* homeobox 6 (Six6) and Six7 are the transcription factors responsible for expression of two middle wavelengths-sensitive opsins, Blue and Green opsins (*PNAS*, 2019). Further analysis identified Foxq2 is the key transcription factor determining the blue cone identity, under Six6/Six7 regulation (*Science Adv.,* 2021).

(ii) Visual transduction mediated by G-protein, Transducin: We found C-terminal farnesylation and carboxyl methylation of Transducin (Gt) gamma-subunit (*Nature,* 1990; *EMBO J.,* 1991). I was interested in physiological significance of the difference between the C15- and C20-modifications. We developed knock-in mice, in which the C-terminal CAAX sequence, CVIS, directing farnesylation (C15) was replaced by CVIL directing geranylgeranylation (C20). Then light-adaptation of the mutant rod cells was significantly impaired because C20-modified Gt was unable to translocate from the outer segment to the inner region upon light illumination (*Neuron,* 2005). We also identified that the *N*-terminus of Gt alpha-subunit is heterogeneously modified by one of myristate and three related fatty acids. It turned out that the heterogeneous *N*-acylation contributes to broadening of the light-sensitive range of the rhodopsin signaling due to functional divergence among the Gt-alpha subspecies (*Nature*, 1992).

(iii) Pineal photoreception and photic entrainment of circadian clock: Dr. Toshiyuki Okano and I found chicken pineal photoreceptive molecule responsible for light-dependent inhibition of melatonin production, and he named the new opsin "pinopsin" after pineal opsin as the first example of opsins expressed in extra-retinal tissues (*Nature,* 1994). Then, we found that photoactivation of pinopsin triggers the phase-shift of the pineal circadian clock through signaling of G-protein G_{11} (*J. Neurosci.*, 2002). We identified *E4bp4* gene as a light-inducible gene regulating the phase-shift of the pineal clock (*Curr. Biol.*, 2004). Then Dr. Kojima and I became interested in similarity in gene expression profiles between the pineal and retinal cells, and we found an important DNA *cis*-element governing the pineal-specificity in gene expression, and named it PIPE after "pineal expression-promoting element" (*PNAS,* 2002). Finally, we identified a homeobox protein Bsx responsible for the development of the pineal gland and pineal-specific gene expression in zebrafish (*Commun. Biol.*, 2019).

2

Light in the life of marine phytoplankton

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Phytoplankton are aquatic microscopic photosynthetic organisms responsible for about half of the photosynthetic activity on Earth. Over the past two decades, breakthroughs in genomics and ecosystem biology have significantly expanded our understanding of the crucial role these microbes play in global ecosystems.

The life of phytoplankton is profoundly influenced by the underwater light distribution, which diminishes in intensity and changes in spectrum with depth. Phytoplankton have evolved effective systems for optimizing light harvesting and energy generation through photosynthesis. However, the role of light sensing in acclimation mechanisms, which control growth and distribution of these phototrophs across different environments, remains poorly understood. To address these gaps, we are conducting integrated analyses of light-driven processes in diatoms, a prominent and highly diverse group of phytoplankton that stand at the crossroads of several evolutionary lineages. Recent functional studies in diatom model species and environmental investigations have identified new regulators of photosynthesis, photoreceptor variants, and a long-sought circadian clock controlling diatom responses to periodic light/dark cycles. Notably, sensors typically responsive to red and far-red light, such as the Diatom Phytochrome (DPH) have been described despite their presence seems counterintuitive in the red-absorbing water column. Our recent research reveals that DPH indeed mediates photoreversible responses across the entire underwater light spectrum, acting as a sensitive detector of optical depth. The observed regulation of photosynthesis acclimation by DPH links the optical depth detection with a relevant physiological response.

These findings provide new insights into how light-driven processes have evolved, diversified, and function in the marine environment. They also highlight the central role of photoregulation in the functional biodiversity of phytoplankton and the importance of integrated laboratory and environmental studies for understanding life in marine ecosystems.

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3

Molecular properties and optogenetic potentials of diverse animal opsins

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Animal opsins are light-sensitive G protein coupled receptors (GPCRs). Thousands of the opsins have been identified from a wide variety of animals and they are phylogenetically divided into several groups. The members belonging to each group have unique molecular properties and the properties could potentially contribute to GPCR optogenetics, namely, optical control of GPCR functions. I discuss optogenetic potentials of animal opsins from the viewpoints of their molecular properties. Most animal opsins require an 11-*cis* retinal as a chromophore, but the amount of the 11-*cis* form in extraocular tissues is extremely limited. Therefore, the demand for the 11-*cis* form has been often pointed out as a disadvantage of animal opsins in optogenetic application. Bistable opsins, which exhibit an interconvertible photoreaction between two stable states, dark-inactive and light-active states, do not release chromophore retinals and therefore, they potentially overcome the disadvantage in optogenetics [1, 2]. In my presentation, I discuss optogenetic potentials about some bistable opsins such as parapinopsin, a typical bistable opsin involved in the pineal wavelength discrimination [1,3], a mutant protein of the jumping spider peropsin that binds to all-trans retinal in the dark and light-dependently stops activating G protein [4] and some opsins including jumping spider Rh1 for activating Gq-mediated signal transduction cascades.

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An ultrafast opsin from the dreaded box jellyfish

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Abstract Content TBC

5

Structure of an active bistable invertebrate rhodopsin: Implications for the mechanistic understanding of bistability

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Abstract content TBC

6

Evolution of the jumping spider rhodopsin and its optogenetic potentials

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Opsins, a member of the G protein-coupled receptor (GPCR) family form photoreceptor proteins by binding the chromophore retinal to underlie photoreception of animals, such as vision [1]. Jumping spiders are highly vision-dependent animals and jump accurately to capture their prey. We previously reported that the measurement of distances to the targets, so-called depth perception, is achieved based on defocused images captured by the second deepest layer of the four-layered retina, where a green-sensitive rhodopsin (Rh1) is localized but green light is not focused [2, 3]. Here we investigated the significance of the spectral sensitivity of jumping spider Rh1 in the depth perception mechanism by analyzing absorption spectra of Rh1s of various jumping spider and “non-jumping” spider species. Mutational analysis of recombinant spider Rh1s with the aid of ancestral sequence inference uncovered the spectral tuning sites for the evolution of the absorption spectrum of jumping spider Rh1. In addition, since optical control of GPCR signaling using bistable animal opsins has recently been developing [4, 5], we also demonstrate the optogenetic potential of jumping spider Rh1, a Gq-coupled bistable opsin, in GPCR signaling-mediated physiological responses in mice.

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Melanopsin, from molecule to behavior

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Melanopsin is a fly-like visual pigment (R-type) expressed in a small subset of light-sensitive retinal ganglion cells (ipRGCs) in the mammalian retina. It is involved in regulating non-image forming visual behaviors, such as circadian photoentrainment and the pupillary light reflex (PLR), as well as aspects of image-forming vision. ipRGCs act as autonomous photoreceptors via the intrinsic melanopsin-based phototransduction pathway and as a relay for rod/cone input via synaptically driven responses. The regulation of melanopsin by phosphorylation profoundly effects its kinetics. C-terminal phosphorylation of melanopsin determines the recovery kinetics of the intrinsic melanopsin-based photo response in ipRGCs, the duration of the PLR, and the speed of reentrainment. RGS proteins are also involved in modulating light-activated melanopsin. Understanding the biochemistry of the visual pigment melanopsin leads to insights into the physiology and behavior associated with ipRGCs.

8

Differential roles of multiple photoreceptors in regulating background adaptation of zebrafish

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Publish consent withheld

9

Novel verteporfin-based nanoparticles for targeted photodynamic therapy of ovarian cancer

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Ovarian cancer is the fifth most frequent cause of death in women, claiming the highest mortality rate within gynecological cancers.[1] The standard of care for advanced-stage ovarian cancer is surgical debulking and platinum-based chemotherapy, followed by maintenance treatment with PARP inhibitors and/or bevacizumab. Despite these, the majority of women face relapse, resulting in a concerning 5-year survival rate of approximately 30%.[2] To mitigate treatment-associated toxicity while augmenting therapeutic outcomes, targeted therapies emerge as promising avenues. Folate receptor (FR) is overexpressed in over 70% of primary and 80% of recurrent ovarian cancers. Additionally, the expression of FR has been shown to remain high after chemotherapy.[3] Photodynamic therapy (PDT) is a minimally invasive treatment in which administration of a light-activated drug is followed by irradiation at a specific wavelength leading to the production of cytotoxic reactive oxygen species.[4] Verteporfin (VP) is a well-known photosensitizer that presents low aqueous solubility and has been approved by the Food and Drug Administration (FDA) as a liposomal formulation.[4]

Leveraging VP's potential and FR specificity, a novel approach (VP-NPs) emerges for the targeted PDT of ovarian cancer. A novel VP-derived molecule was first developed to enhance encapsulation efficiency into nanoparticles. Cellular uptake studies were conducted in FR-positive ovarian cancer cells, and phototoxicity was assessed upon irradiation. Biocompatibility assays were performed to evaluate the safety profile of VP-NPs in vitro. VP-NPs exhibited increased photoactivity, singlet oxygen production and biocompatibility relative to the VP-derived molecule alone. Furthermore, VP-NPs demonstrated superior PDT efficiency and showed increased cellular uptake by FR-positive ovarian cancer cells compared to non-targeted nanoparticles. Our study highlights the potential of VP-NPs as a targeted PDT agent for ovarian cancer therapy, offering improved biocompatibility, specificity, and therapeutic efficiency. These findings lay the groundwork for further investigation and potential clinical application of VP-NPs in ovarian cancer treatment.

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10

Polydopamine-Based Nanophotosensitizing Systems for Targeted Phototherapy

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Polydopamine (PDA) is a promising biomaterial derived from the self-polymerization of dopamine under alkaline conditions. With various reactive functional groups on the surface, PDA supports facile modification with thiol- and amine-containing molecules and coating on different types of materials. Owing to the biocompatibility, high cellular uptake, low cytotoxicity, and fluorescence

quenching ability, PDA-based nanomaterials have found a wide range of biomedical applications, such as bioimaging, molecular diagnostics, gene delivery, controlled drug release, photothermal therapy, cancer theranostics, and antimicrobials. However, their limited biodegradability greatly obstructs their clinical translation. We have recently developed a series of polydopamine-based nanosystems with thioketal, disulfide, or diazo linkages, which are cleavable by reactive oxygen species, thiols, and reducing agents, respectively. These biodegradable nanoplatforms can be used to encapsulate a chemotherapeutic drug or photothermal agent, immobilize a photosensitizer, and incorporate a tumor-targeting peptide to form tumor-directed and stimuli-responsive nanodrugs for synergistic chemo and photodynamic therapy against cancer. The results will be reported and discussed in this presentation.

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Targeted photo-activable multi-agent liposome for fluorescence-guided photoimmunotherapy enhances survival outcomes

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Peritoneal metastasis is the result of direct shedding of tumor cells from ovarian, colorectal, and other cancers that lead to studding and growth on the peritoneal surfaces and abdominal organs. Standard chemotherapy and surgery can transiently improve symptoms, but long-term disease control and survival extension remain rare due to residual disease and treatment resistance. In the past 40 years, tremendous progress in photoimmunotherapy (PIT) has been made to treat metastasis. However, many are lost in translation due to heterogeneity in photosensitizer drug uptake and treatment response. Therefore, it is critical to develop targeted therapies with a multimodal approach that integrates well with current standards of care strategies and overcomes heterogeneous delivery and treatment responses. Our novel targeted photo-activable mult-agent liposomal platform combined with fluorescence-guided PIT has demonstrated its capabilities to overcome these challenges in our prior work and we further demonstrate its efficacy by studying (1) tumor nodule penetration depth, (2) tissue optical property changes pre and post multi-dose PIT, and (3) enhanced long term therapeutic efficacy compared to monotherapy treated groups in a mouse peritoneal carcinomatosis model.

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Light-Activatable, Sustained-exposure Ethanol Injection Technology (LASEIT) for treatment of locally advanced tumors

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Each year, over 1 million patients die from pancreatic and liver cancer worldwide. Surgery and transplantation are the only curative modalities for these cancers; however, less than 30% of patients are eligible for surgery. New treatments are needed to convert non-surgical to surgical candidates. Photodynamic therapy (PDT) is an emerging modality for the treatment of pancreatic and liver cancers. Currently, most photosensitizer drugs are being delivered through intravenous injection or oral administration, and low percentages of administered dose reaches the tumor. We have developed a Light-Activatable, Sustained-exposure Ethanol Injection Technology (LASEIT) that can improve the efficacy and safety of PDT for locally advanced, unresectable tumors. We have demonstrated that LASEIT enhances photosensitizer delivery by up to 40X and improves light propagation in the tumor. These features can significantly reduce side effects, shorten operating room turnover time, and improve PDT efficacy.

13

Natural photoenzymes

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Until the end of last century, the natural photoenzymes known were (i) Photosystem I and II as a unit, photocatalysing water decomposition into O_2 and H^+, (ii) protochlorophyllide reductase photocatalysing chlorophyllide production (with NADPH as cofactor) and (iii) photolyases as DNA repair enzymes in many organisms (except mammals) with a reduced flavin adenine dinucleotide ($FADH^-$) as chromophore and a second flavin or a pterin as a second cofactor.

Since the beginning of the new millenium several biological photoreceptors have been characterized as photoenzymes. Among those containing a flavin as chromophore, adenylyl cyclases[1] are found in microorganisms and photodercaboxylases in algae.[2] In other microorganisms enzymerhodopsins act as kinases, cyclases or phosphodiesterases, all of them with retinal as chromophore.[3]

Photosensors containing an open-chain tetrapyrrole chromophore have also being characterized as photoenzymes. Microbial phytochromes act as light regulated histidine kinases or phosphatases in two component systems[4] and cyano-bacteriochromes in cyanobacteria are adenylyl cyclases.[5] It has also been shown that eucaryote phytochromes are protein kinases as well as autophosphorylation kinases.[6],[7]

The diversity of chromophores in different protein matrices and the knowledge about the function of the natural photoenzymes offer multiple possibilities to mimic the biosystems and design photoenzymes excitable with a wide range of wavelengths to

perform different, non-natural tasks, with ecological advantages, e.g., by replacing chemical reactions in organic solvents with metal-containing catalysers, extensively used in the chemical industry.

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14

Fatty Acid Photodecarboxylase (FAP): a gateway to non-fossil hydrocarbon fuels and beyond...

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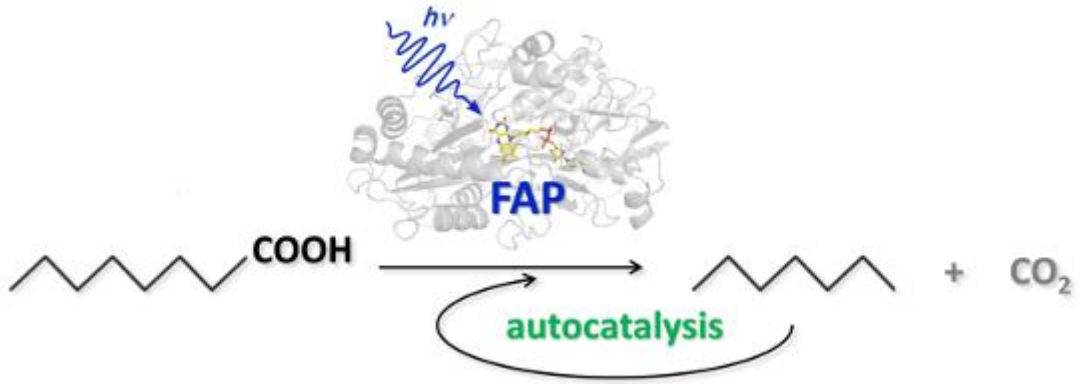
Addressing the urgent need for renewable, carbon-neutral, and locally sourced alternatives to fossil fuels amid ongoing climate change and geopolitical tensions, the quest for green fuels intensifies. Photocatalytic conversion of fatty acids to hydrocarbons by Fatty Acid Photodecarboxylase (FAP)[1,2] emerges as a promising pathway. Despite prior concerns about its efficacy on C2-C12 fatty acids, recent findings challenge this assumption. Our results[3] unveil that *Chlorella variabilis* FAP (*Cv*FAP) demonstrates significantly enhanced activity, converting octanoic acid four times faster than hexadecanoic acid, its best substrate reported to date. Moreover, *in vivo* experiments reveal a remarkable ten-fold increase in *Cv*FAP-based production rates for n-heptane compared to n-pentadecane. Time-resolved spectroscopy and molecular modeling shed light on the underlying mechanism, revealing an autocatalytic effect of n-heptane contributing to the high catalytic activity of FAP on octanoic acid[3]. These insights pave the way for future FAP enhancement strategies, marking a crucial milestone towards the bio-based, light-driven production of gasoline-like hydrocarbons.



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15

Enhanced light-driven DNA repair by a photolyase bearing an artificial light-harvesting chromophore

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Photolyases are flavoenzymes responsible for the repair of carcinogenic DNA damage caused by ultraviolet radiation. They non-covalently harbor a catalytic cofactor flavin adenine dinucleotide (FAD). The blue light-driven electron transfer from the excited

state of the fully-reduced form of FAD to the DNA lesions induces rearrangement of the covalent bonds, leading to the restoration of intact nucleobases. Since DNA repair by photolyases is independent on other DNA repair pathways, it has been considered as a potential molecule for gene therapy of diseases related to repair of UV-induced DNA damage. However, permeability of blue light to organs is low. If the DNA repair activity of photolyases can be artificially enhanced, such an artificial DNA repair system will be applicable to the gene therapy.

In addition to the catalytic chromophore, some photolyases contain a secondary chromophore with better light absorption capability than FAD, acting as a light-harvesting chromophore that harvests photons in sunlight efficiently and transfers light energy to the catalytic center, as observed in natural photoreceptor proteins. Inspired by nature, a synthetic fluorescent chromophore was attached to the surface of photolyase using oligonucleotides containing a modified nucleoside and a cyclobutane-type DNA lesion. The modified enzymes successfully enhanced its enzymatic activity in the light-driven DNA repair. This first-generation strategy gave us a clue for appropriate amino acid side chains to be modified, and therefore site-selective conjugation of fluorophores was performed. The results indicate that one of the fluorophore-modified enzymes exhibited further enhancement of the activity.

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Photoswitching of Flavin–Inhibitor Complexes in Flavoenzymes

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The vast majority of flavoenzymes, including monomeric sarcosine oxidase (MSOX) and *N*-methyltryptophan oxidase (MTOX), perform non-light-driven physiological functions.[1] However, the involvement of flavin cofactors in photoinduced reactions is widespread in nature.[2] MSOX and MTOX both harbor an oxidized flavin adenine dinucleotide (FAD_{ox}) cofactor, and catalyze the oxidative demethylation of sarcosine and *N*-methyltryptophan, respectively. Methylthioacetate (MTA) and methylselenoacetate (MSeA) are substrate analog inhibitors of MSOX that form complexes with MSOX, exhibiting intense absorption bands over the whole visible spectral range due to flavin−MXA (X = T, Se) charge-transfer (CT) interactions (Fig. 1a). Based on femtosecond transient absorption (TA) measurements, we show that when the MSOX:MXA complexes are photoexcited, the CT interactions disappear during a barrierless reaction in ca. 300 fs with a high quantum yield (Fig. 1d). The initial complex subsequently geminately reforms in a few nanoseconds near room temperature in a thermally activated way (Fig. 1b).[3] In the experimental crystal structure of MSeA-containing MSOX, the ligand is bound to the protein in two discrete conformations (Fig. 1c; ***Conf 1*** vs. ***Conf 2***). Considering the timescales of the reactions, it is highly plausible that on the molecular level, the switching of the FAD_{ox}:MXA complexes involves the isomerization of MXA between ***Conf 1*** and ***Conf 2***.[4]

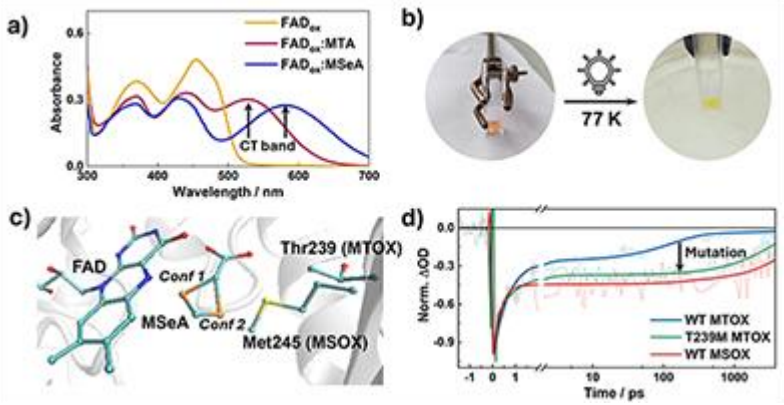

Fig. 1. (a) Spectra of the FADox:MXA complexes in MSOX. (b) Photoswitching of the FADox:MTA complex in MSOX at 77K. (c) Active-site structures of MSOX and MTOX containing MSeA in two conformations. (d) TA kinetics of the MSOX and MTOX variants complexed with MSeA pumped at 550 nm and probed at ca. 600 nm.

By contrast, in MTOX, which can also bind MSeA to form a CT complex, we did not observe efficient photoswitching of the FAD_{ox}:MSeA complex upon excitation, with a slower forward switching (ca. 700 fs) and a much faster back recovery (ca. 140 ps). As a close homologue of MSOX, MTOX has an active site that highly resembles that of MSOX in structure, with the exception that a methionine residue, Met245, in MSOX, closely interacting with the MSeA ligand in ***Conf 2***, is replaced by a threonine (Thr239) in MTOX, located farther away from the ligand (Fig. 1c). This indicates that the presence of the nearby Met245 is a prerequisite for efficient photoswitching, which is corroborated by the experiment on the T239M mutant of MTOX, where the threonine-to-methionine mutation significantly activates the photoswitching, slowing down the back recovery to a nanosecond timescale (Fig. 1d). Molecular dynamics simulations and quantum chemical calculations provide detailed insights into the interactions between FAD_{ox} and MXA in the protein active sites, and demonstrate the effects of Met245 on the conformations of MXA and the energetics involved.[5] Conformational photochemical processes in CT complexes may be further explored for novel photocatalytic and photoswitching applications of flavoproteins.

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17

Making the sunshine vitamin – how much sun exposure is needed to maintain 25 hydroxy vitamin D concentration?

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Background: Advice about how to balance the risks and benefits of sun exposure, and the time needed to avoid vitamin D deficiency, varies between organisations. In light of this, the purpose of this study was to calculate the time in the sun necessary to maintain existing 25(OH)D concentration throughout the year, at various locations across Australia and New Zealand, with different clothing coverage1. This information is critical to informing public health policy when considering the risks and benefits of sun exposure.

Methods: We used a microsimulation model2 to estimate changes in monthly 25(OH)D concentration using data on standard erythemal dose, solar zenith angle and climatological ozone. We estimated the number of standard vitamin D doses per 10 minute interval, and used a dose-response equation to determine the minimum time in the sun to maintain existing 25(OH)D concentration according to month and time of day

Results: Across all locations in summer, 5-10 minutes outdoors between 8am and 4pm on most days of the week, with 35% of the body surface area exposed, is sufficient to maintain existing 25(OH)D concentration. In winter, at mid-to-high latitudes, time outdoors during the middle of the day is required.

Conclusion: Provided sufficient skin is exposed, a small amount of time outdoors will maintain existing 25(OH)D concentration across most of Australia and New Zealand, with the exception of higher latitude locations in winter. These data can be used to inform guidelines regarding maintaining vitamin D via sun exposure, and may help health practitioners identify patients who may be vitamin D deficient. This presentation will also present the findings on the expected health and healthcare costs if sub-populations amended their sun exposure to optimise health outcomes for five sun-related conditions; melanoma, keratinocyte skin cancer, cataract, multiple sclerosis and fragility fractures.

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How does natural sun protection and sunscreen use influence vitamin D synthesis?

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Terrestrial solar UVB radiation (~295-315nm) drives cutaneous vitamin D synthesis but is also the main cause of erythema (sunburn) with overlapping action spectra for both endpoints. UVB converts 7-dehydrocholesterol (7-DHC) in skin into pre-vitamin D, which then undergoes UVR independent steps to become functional vitamin D. People with highly pigmented skins typically have poorer vitamin D status that those with lighter skins within a given latitude band. The inhibitory effect of melanin has not been quantified. A comparison of Fitzpatrick skin type (FST) VI (black) versus II (white) showed an inhibitory factor <1.5 which is less than melanin's ability to inhibit sunburn [1]; the minimal erythema dose (MED) for FST VI is about 7-10 times greater than those with light skin.

UVB attenuation by sunscreens to prevent sunburn would be expected to inhibit vitamin D synthesis. Carefully monitored field studies show that correct sunscreen use (labelled sun protection factor (SFF) of 15), inhibited sunburn, and to some extent vitamin D synthesis, but allowed considerable enhancement of vitamin D status during a one-week holiday in which peak UV index was very high [2]. This is because sub-erythemal exposure is sufficient for good vitamin D synthesis. We estimated that the holidaymakers received about $1/10^{th}$ of an MED per day through the sunscreen.

In summary, melanin and sunscreens are chromophores that attenuate UVB radiation. Melanin affords protection against erythema with a "SPF" of about 7-10 but has a modest inhibitory effect on vitamin D synthesis. The most likely reason for this is that there is sufficient 7-DHC above the melanin rich basal epidermis to enable vitamin D synthesis. In the case of sunscreens, the dose threshold for vitamin D synthesis is much lower than that for erythema. The lessons from these studies are that chromophore location is important and different end points have different UVR dose thresholds. They also show that sunscreen use (SFF 15) does not compromise vitamin D synthesis. We lack data on high SPF (50+) sunscreens.

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Protection of vitamin D compounds against UV-induced skin carcinogenesis

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Australia has one of the highest rates of skin cancer globally, with two in three people being diagnosed by the age of 70. Whilst non-melanoma skin cancers are more common, melanoma is responsible for the majority of skin cancer-related deaths. The main contributors to ultraviolet radiation (UV)-induced skin carcinogenesis are UV-induced DNA damage, some of which is inadequately repaired, resulting in mutations, and UV-induced immune suppression, which results in failure of recognition and elimination of developing skin tumours by immune surveillance. Another consequence of UV is the synthesis of vitamin D and its active metabolite, 1,25-dihydroxyvitamin D3 (1,25D) in skin cells. Epidemiological studies have linked vitamin D status to melanoma risk and outcome. Our studies have shown that the active vitamin D metabolite 1,25D and related compounds including 1,25-dihydroxylumisterol and tetrahydrocurcumin can inhibit UV-induced DNA damage, immune suppression and skin carcinogenesis. Phosphatase and tensin homolog (PTEN) and N-myc downstream regulated gene-1 (NDRG1) are proteins that are lost or suppressed during carcinogenesis and metastasis. We showed that levels of both these proteins are significantly reduced 24 h after UV in primary human skin cells and in Skh:hr1 mouse skin, but significantly increased with 1,25D treatment. We have also shown that 1,25D can increase levels of proteins that play a key role in nucleotide excision repair of UV-induced DNA damage. Our studies suggest a role for vitamin D compounds in the prevention of skin carcinogenesis and inhibition of melanoma cell growth. Vitamin D-like compounds that are less calcemic but photoprotective are promising for use in after-sun lotions to protect against acute UV damage while also preventing skin carcinogenesis.

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New action spectrum for vitamin d production in human skin – Does this alter the risk-benefit balance?

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Background: Ultraviolet (UV) radiation of human skin causes DNA damage, mainly cyclobutane pyrimidine dimers (CPDs), but also have the beneficial effect of vitamin D3 synthesis. The production of vitamin D is dependent on wavelength and can be described by weighting functions called action spectra. Current public health advice on optimal vitamin D status maintenance is partly based on the CIE pre-vitamin D actions spectrum, but this is under debate.

Aim: To simultaneously determine quantitative action spectra of vitamin D3 and CPD in human skin, obtained under identical exposure regime.

Materials and Methods: We have obtained excess waistband skin from 3 persons just after it was surgically removed. From each person's skin tissue 82 biopsies were prepared: 80 irradiated with one of 10 UV-LEDs with wavelengths from 280 to 335 nm and 2 non-irradiated controls. Half the biopsies were quantified for vitamin D3 by UHPLC-MS/MS, the other half were quantified for CPDs in the skin by HPLC-MS/MS. For each wavelength, 4 doses with linear increments were given and a linear dose response was calculated. The regression slopes are presented as action spectra.

Results: Both vitamin D3 and CPD action spectra have the maximal peak at 290 nm with a decrease towards higher wavelengths. In the interval 295-310nm the normalized action spectra of vitamin D3 are 1.4 to 1.7 times higher than the CPD action spectra otherwise the CPD action spectra are 1.3-10 times higher. From 300 to 315 nm vitamin D3 production is relatively lower than the CIE pre-vitamin D3 action spectrum.

Conclusions: We find that that the CIE pre-vitamin D actions spectrum overestimates the vitamin D production after UVB exposure, which is in accordance with part of the literature debates.

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Phycobilisome assembly and attachment to photosystem II for energy conversion in cyanobacteria

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Phycobilisomes are the major light harvesting complexes in photosynthesis. Two types of phycobilisomes exist: the large PBS consisting of a core and rods (herein PBS) and the rod-only CpcL-PBS, which contains a membrane-attaching linker CpcL. PBS that are mainly associated with PSII and CpcL-PBS are mainly associated with PSI. We here report (1) the Cryo-EM structure of PBS from *Synechococcus* sp. PCC 7942, which contains a two-cylinder core and six rods. The bundle-shaped PBS structure of *Gloeobacter* 7421 was also determined with Cryo-Em combined with mutagenesis analysis. (2) We compare how rods are attached to the cores in different cyanobacterial PBS, which are crucial to the functions of PBS. (3) The roles of linker proteins as well as phycobiliproteins in energy transfer in PBS. (4) The attachment of PBS to PSII is studied and we show here that a small linker protein (Linker for PBS-PSII, Lpp72) is required for such an attachment in the cyanobacterium *Synechococcus* PCC 7002. In the absence of the gene encoding Lpp72, *Synechococcus* 7002 grew much more slowly under a green light illumination. Oxygen evolution rate in the lpp72 mutant illuminated with a 590-nm light was greatly reduced. We demonstrate that Lpp72 interacts with both CP47 of PSII and ApcB of PBS.

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Photoisomerization Mechanism of Retinal in Different Rhodopsins - Insight from Multiscale Simulations

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Rhodopsins are light-sensitive, membrane-embedded proteins which bind a retinal as a chromophore. They have different functions and are found in various organisms. However, they all have the initial chemical reaction in common, namely the photoisomerization of the retinal chromophore. This process involves an isomerization of a double bond upon illumination with light. The initial and the product conformations of the retinal depend on the rhodopsin. In visual rhodopsin (also known as type II) the protein binds 11-*cis* retinal which isomerizes to the all-*trans* conformer. However, in microbial rhodopsin (also known as type I) the protein binds retinal in the all-*trans* conformation, which isomerizes to 13-*cis* isomer.

However, this isomerization categorization was recently challenged with the discovery of the fusion of bestrophin and rhodopsin, the so-called bestrhodopsin.[1] It was reported to isomerize all-*trans* retinal to 11-*cis* instead of the typical 13-*cis* isomer. In contrast, a recent ultrafast Raman experiment has assigned the initial product to 13-cis conformer.[2]

In this contribution we will present results from the computational studies which use the cryo-EM structure as a starting point and utilize non-adiabatic dynamics with the hybrid quantum mechanics/molecular mechanics (QM/MM) method. These results provide atomistic insight into the mechanism and reveal the critical role of the protein environment.

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Ultrafast primary dynamics and isomerization mechanism of a far-red sensing cyanobacteriochrome

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Far-red cyanobacteriochromes (CBCRs) are newly discovered bilin-based photosensory proteins that promise to offer novel optical agents in optogenetics and deep tissue imaging. Recent structural studies of a far-red CBCR 2551g3 have revealed a unique *all-Z,syn* chromophore conformation in the far-red-absorbing Pfr state. Understanding the photo-switching mechanism through bilin photoisomerization is important for developing novel biomedical applications. Here, we employ femtosecond spectroscopy and site-directed mutagenesis to systematically characterize the dynamics of the wild-type 2551g3 and four critical mutants in the 15Z Pfr state. We captured local relaxations in several picoseconds and isomerization dynamics in hundreds of picoseconds. Most mutants exhibited faster local relaxation while their twisting dynamics and photoproducts depend on specific protein-chromophore interactions around the D-ring and C-ring. These results collectively reveal a unique dynamic pattern of excited-state evolution arising from a relatively rigid protein environment, thereby elucidating the molecular mechanism of Pfr-state photoisomerization in far-red CBCRs.

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Light Signaling and Allostery Mechanisms of Bacteriophytochromes

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Phytochromes are a superfamily of bilin-based photoreceptors that mediate a wide range of light responses in plants, fungi and bacteria. In photosynthetic bacteria, they regulate gene expression of key photosynthetic components and pigment-processing enzymes. Canonical bacteriophytochromes (BphPs) are multi-domain sensor histidine kinases that undergo light-dependent auto-phosphorylation in a two-component system where the phosphoryl group is relayed to an Asp residue in a cognate response regulator, thereby triggering downstream transcriptional actions. Despite the extensive studies on bacteriophytochromes, the molecular mechanisms of light signaling and allostery remain elusive in the absence of full-length structures representing distinct signaling states. To address this challenge, we tackle a few representative BphPs using an integrated approach of biochemistry, spectroscopy, mutagenesis and structural biology. Specifically, we harness dynamic crystallography and single particle cryo-electron microscopy to provoke, probe and resolve the functional relevant structural dynamics in the truncated photosensory domains and full-length proteins. Findings are expected to elucidate the long-range signaling mechanisms in dimeric receptor kinases beyond photosynthesis and photoreceptors, which also promise to offer optogenetic solutions for biomedicine and renewable energy research. In this conference, I will present our recent cryoEM studies of BphPs that undergo large structural changes in response to light.

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Light Intensity-Dependent Photo-Activation Quantum Yield of Orange Carotenoid Protein

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Orange Carotenoid Protein (OCP) is a unique photoactive protein containing a carotenoid as the photo-responsive chromophore, involved in cyanobacterial photoprotection. The photo-activation of OCP begins with the picosecond evolution of the ketocarotenoid excited-state levels and involves structural changes occurring up to second time scale, ultimately leading to the active red OCP with a quantum yield of about 0.2%. There is a longstanding debate on the origin of the red OCP's low quantum yield and the intermediates that control it. Additionally, reports published so far implicitly assume that the absorption of only one photon is enough to initiate complete photoconversion to the active OCP form.

The major challenge in deducing the photoactivation mechanism is creating a uniform explanation for both single-pulse excitation experiments and continuous light irradiation experiments. We studied the photodynamics of different OCPs and carotenoids using time-resolved X-ray scattering and Visible-NIR transient absorption spectroscopy, considering variables such as concentration, His-tagging, excitation pulse power, and wavelength. Additionally, we performed nanosecond to second time resolved transient absorption experiments coupled with stationary irradiation light to verify the single-photon hypothesis.

Our experiments showed a light intensity dependence of the photo-activation yield. These results clearly demonstrate that single-photon absorption cannot explain the observed dynamics of OCP under biological irradiation conditions encountered in vivo. We will then discuss here the entire photo-activation mechanism of OCP, from the femtosecond to second time scale, with a special focus on the light intensity response.

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Development of organic photo-drugs for the treatment of cancers

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Photodynamic therapy (PDT) is a clinically approved, noninvasive cancer treatment that involves administering a photosensitizer (PS) and light to the affected area. The current range of photodynamic therapy agents is limited, and there is a pressing need for cost-effective, organic photosensitizers that can offer improved efficacy under multiple photosensitization mechanisms. In this talk, I will present some of the recent advances made by our group in developing biocompatible all-organic PSs that exhibit tunable absorption spectra from the ultraviolet-A (UVA) to the near-infrared (IR) regions of the electromagnetic spectrum. Several of these PSs exhibit excellent PDT efficacy against monolayers of human epidermoid carcinoma, melanoma, cervical, and human epithelium cancer cells, regardless of the oxygenation status (i.e., under both normoxic and hypoxic conditions), when applied in vitro with a low dose of light.

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Phototoxicity of retinoid drugs: from photophysics to photobiology

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Since their introduction in the 1980s, retinoids have been increasingly used for topical and systemic treatment of skin conditions such as acne-related dermatoses or psoriasis but also for therapy and/or chemoprevention of skin cancer and other neoplasia. Along the years, chemical modifications have led to more efficient and safer drugs. But, from a photobiological viewpoint, development of the 3rd generation of polyaromatic retinoids calls the attention on their potential photoactivity. In this context, adapalene along with tazarotene and tretinoin are currently the three topical retinoids approved so far by the Food and Drug Administration (FDA).

Therefore, a detailed study of their photophysical and photobiological properties is critical to evaluate their photoreactivity toward biological components and their potential use as phototherapeutic agents. In a first stage, the photophysical properties of these drugs are investigated to get more insight into their excited states and their potential to trigger biomolecule damages. Then,

retinoids phototoxicity is established *in vitro* using the standard Balb/c 3T3 NRU assay, that reveals a photoirritation factor (PIF) higher than the threshold of 5 set by the guidelines for phototoxic compounds. The Type II reactivity of these topical drugs is discussed together with their potential activity and use as phototherapeutic agents.

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Photostability of anticancer monoclonal antibodies

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Monoclonal antibodies (mAbs) are complex protein molecules, and their structural integrity impacts on their biological and pharmacological activity.

We investigated the effect of some stress factors, i.e., shaking, temperature, dilution, and light, on formulated anticancer monoclonal antibodies Nivolumab (Opdivo®) and Cemiplimab (Libtayo®**)** with or without dilution (saline and glucose solutions) trying to mimic their routine handling once released from the pharma industry, shipped to the hospital, diluted for the parenteral administration, and finally administered to the patients.

mAb stability analyses were run through biochemical and biophysical methodologies but a surface tensiometry analysis was added to get new information on the mAb chemico-physical changes induced by light. Indeed, a new concept of Integrated Analytical Approach was applied to the study of solid/liquid complex systems, determining the contact angle at the interface between the liquid samples and the perfluoropolyether liquid film as a "solid substrate", without the influence of s/l interfacial friction forces and roughness surface.

Both mAbs showed to be quite stable under shaking and moderate temperature (37°C) for 45 days. However, they underwent chemico-physical modifications, mostly aggregation and amino acids oxidation, upon exposure to artificial sunlight. The light treatment produced major variations in surface tension of mAb+NaCl respect to mAb+glucose since aggregates influence surface tension properties. No significant effects to the secondary and tertiary structures were detected.

The dilution media commonly used for the administration to the patients, particularly the sterilized glucose solution containing degradation glucose products, i.e., HMF, showed a remarkable impact on the photostability of these drugs. Biological tests on the photomodified mAb gave information on the residual activity of the drug.

The instability of mAbs in sterile glucose solution upon light exposure could have a negative effect on the safety and efficacy of these very active anticancer drugs and should be considered in their real-life.

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Photo(un)stability of drugs and biomarkers

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Pharmaceutical drugs, drugs of abuse and/or their metabolites are emerging environmental pollutants. The identification of their degradation/photodegradation processes is of interest in the so called "sewage epidemiology," for estimating drug consumption in a community by wastewater analysis, and in toxicity studies for understanding their toxic effects in the aquatic environment.

The UVA and UVB light-induced behaviour of 6-monoacetylmorphine (6-MAM) and morphine, the two main metabolites of heroin, was studied in methanol, aqueous solution and in the dry state. Irradiation was performed at 365 (UVA) and 312 (UVB) nm, for different times, achieving radiant energies of 20-300 J/cm^2. UV spectra of irradiated samples, at selected dosages, were compared with the spectra of the same samples kept in the dark. In order to estimate the extent of photolysis, positive ion electrospray ionization experiments were performed on the irradiated samples by High Resolution, High Accuracy Mass Spectrometry (HRMS) and by liquid chromatography-HRMS. Tentative identification of photoproducts was performed on the basis of their elemental formula as calculated by HRMS results. Morphine and 6-MAM demonstrated to be quite stable under UVA light but very sensitive to UVB irradiation. The production of singlet oxygen was also evidenced under UVB exposure. In methanol solutions, they undergo a similar pattern, both reaching 90 % photodegradation after 100 J/cm^2 of UVB irradiation, with a slightly faster kinetic for morphine at lower doses. In water, the yields of photodegradation are nearly one third lower than in methanol. In the solid state, the yields of photodegradation is lower than those in solution. The structures of some UVB-induced degradation products, based on HRMS measurements, are proposed. Photoaddition of the solvents and photooxidation seem to be the main pathways of phototransformation of the two molecules. Cocaine and metabolites were studied in an analogous way. Solar light was demonstrated to alter molecular structures of drugs when irradiated in solutions.

In human hair, where drugs of abuse are known to accumulate with chronic use and do not undergo metabolic degradation, drug concentrations are affected by cosmetics or chemical treatments (dying, perming or bleaching) as well as by environmental exposure. Solar light was demonstrated to alter molecular structures of drugs when irradiated in hair, decreasing the concentrations of drugs and/or producing new compounds/metabolites. Studies were performed for different classes of compounds and metabolites by exposure of true positive hair to controlled UVA and UVB light (cocaine, opiates, methadone) or to the whole sunlight spectrum (cannabinoids, cocaine, ethylglucuronide)

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What makes the strength of very phototoxic photosensitizers

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Higher phototoxicity in vitro is readily associated with photosensitizers with higher yields of generation of reactive oxygen species (ROS) in cell media. Although photosensitizers that generate high yields of ROS, notably high singlet oxygen quantum yields (F_Δ), tend to be more phototoxic to cells, cell uptake and subcellular localization are also important determinants of phototoxicity. For example, atropisomers of redaporfin with nearly identical F_Δ have phototoxicities that differ by more than one order of magnitude due to differences in cell uptake.[1] In this work, we explore eukaryotic cell uptake of porphyrins that are precursors of photosensitizers known for their high in vitro phototoxicity. These include porphyrin precursors of Foscan® (IC50=200 nM @ 1J/cm2 for CT26 cells),[2] of IC-H-Me^{2+} (IC50≈500nM @ 5J/cm2 for HEK cells),[3] and of a new bacteriochlorin named LUZ51B (IC50=6nM @ 1J/cm2 for CT26 cells).[4] IC-H-Me^{2+} is a chlorin very effective in the photoinactivation of gram-positive and gram-negative bacteria, and viruses (e.g., SARS-CoV-2). LUZ51B is a carboxamide bacteriochlorin phototoxic to cancer cells and gram-positive bacteria.

This work aims to elucidate the underlying mechanisms that contribute to the phototoxicity of photosensitizers in diverse applications. We report the cellular uptake of the previously mentioned porphyrin precursors of photosensitizers with widely different molecular structures in the JEG-3 cell line. Additionally, we report the uptake of porphyrins lacking therapeutic effects. These porphyrins were selected from a series employed in a screening of antivirals for the Zika virus that included a variety of molecular sizes, charge distributions and functional groups. The screening of antivirals that prevent replication in infected cells, offered clues to differences in cell uptake and to therapeutic efficiency that are discussed in this work.

Acknowledgments

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From photoreceptor light damage to optogenetic and sonogenetic visual restauration

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Light allows vision but it can also trigger blindness. Light is an identified risk factor for the induction of age-related macular degeneration (AMD). In AMD, blindness results from the loss of photoreceptors, but blindness can also occur from the degeneration of retinal ganglion cells forming the optic nerve as in glaucoma.

We have defined the most toxic wavelengths for retinal cells while investigating novel strategies for restoring vision in blind patients.

Using 10 nm bands of wavelengths, we demonstrated that blue violet light is the most toxic light for photoreceptors and retinal epithelial cells. Paris summer sunlight is already in the damaging range. After evaluating an infrared photovoltaic prosthesis in non-human primates, the PRIMA prosthesis reached clinical trials in blind patients affected by AMD. They recovered a visual acuity close to 1/20 allowing word reading. To reach a cellular resolution, we introduced the microbial opsin, ChrimsonR-tdTomato, in retinal ganglion cells. This strategy provided a high spatial resolution with possible video rate activation of the non-human primate retina. Patients injected with the AAV2-7m8 ChrimsonR-tdTomato were able to grasp and count objects on a table. For cortical visual restoration, ultrasounds can penetrate deeply into the brain by contrast to light. Therefore, we proposed to render cortical neurons sensitive to ultrasounds by expressing the mechanosensitive ionic channel MscL via gene therapy. We

provided the proof of concept on rodents that this sonogenetic therapy offers a spatial and a temporal resolution compatible with visual restoration.

In conclusion, we should protect ourselves from the toxic blue violet light. Clinical trials have demonstrated the efficacy of the photovoltaic PRIMA retinal prothesis in blind AMD patients while optogenetic therapy (GS030) was shown to restore partial vision in blind patients affected by retinitis pigmentosa. Sonogenetic therapy offers great hopes for patients with optic nerve atrophy.

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UV carcinogenesis-update-

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Previously UV carcinogenesis has been considered to be cauaed by the accumulation of genetic mutations in cancer-related genes, including oncogenes and tumor suppressor genes such as *p53* genes. Researchers have demonstrated that these genetic changes found in skin cancers of sun-exposed body sites are induced by UV, based on the findings that transition type (pyrimidine to pyrimidine or purine to purine) base changes at di-pyrimidine sites are predominated. However, recent finding revealed that UV carcinogenesis includes more complicated process. Especially, an importance of inflammation is emphasized. It became evident that DNA damage causes inflammation. We have shown that in *Ogg*1 knockout mice, which fail to repair 8-OxoG, manifest much higher frequency of UV-induced skin cancers without the increase in *p53* mutations, and gene expression analysis in this system revealed that the presence of 8-oxoG upregulates inflammatory pathway genes. Further, we have shown that the mice model of xeroderma pigmentosum (XP), DNA repair disorder, where patients manifest severe sunburn and high frequency of skin cancers in the sun-exposed body sites, expressed extremely high level of CXCL1, inflammatory chemokine, and to our surprise, inhibiting CXCL1 using neutral antibody markedly decreased the development of UV induced skin cancers, without repairing dipyrimidine photoproduts. In addition, some anti-inflammatory medicine demonstrated the inhibitory effect of developing UV induced skin cancers. Recently the influence of some medicine in clinical use on UV carcinogenesis has been indicated. For example, some epidemiological studies suggested that the use of voriconazole and hydrochlorothiazide at higher dose for longer period might upregulate UV carcinogenesis process. We have experimentally shown the increase in the formation of DNA damage as well as cytokine expression in the presence of hydrochlorothiazide in mice irradiated with UV. In view of UV sources, many newly emerging UV devices are used in our circumstances. For instance, 222 nm-UV drew our attention during the COVID19 pandemic, because of its shallow reach to the surface of the microorganism and failure of penetrating through its cytoplasm into the nucleus. Although information of safety of far-UVC has not been fully accumulated, as far as UV carcinogenesis studies, 222 nm UVC did not produce any skin cancers even in the XP model mice. However, we need to investigate from various view points on the safety of newly emerged UV devices before its use for human.

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Impact of solar ultraviolet radiation and visible light on human skin

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Ultraviolet radiation (UVR, 290-400 nm) and visible light (VL, 400-700 nm) have significant impacts on human skin, influencing both its health and appearance. UVR, particularly UVB and UVA, can cause photodamage including sunburn, premature aging, and an increased risk of skin cancers. UV exposure can lead to the production of free radicals and DNA damage within skin cells, compromising their function and structure over time. VL, though less energetic than UVR, can also impact skin health by generating reactive oxygen species and inducing pigmentation and erythema in those with dark skin. These VL responses were shown to be potentiated in combination with long wavelength UVA1 (370-400 nm) which human skin is exposed to even after applying organic broad-spectrum sunscreens. These have implications on skin hyperpigmentation disorders such as melasma and post-inflammatory hyperpigmentation, most commonly seen in those with skin of color, and should be considered when counselling patients on sun protection strategies.

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Photodermatoses in Skin of Color

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Photodermatoses encompass a range of skin conditions that are triggered or exacerbated by exposure to sunlight. These conditions may be classified into the following categories: immune-mediated or photodermatoses, chemical- and drug-induced photosensitivity, photoaggravated dermatoses, and others. Depending on an individual's skin type, photodermatoses can vary in occurrence and manifest differently, with skin of color exhibiting unique patterns and challenges due to the interplay of genetic, environmental, and cultural factors. Because of the more subdued erythema and propensity for diseases to present as hyperpigmentation rather than acute inflammation, diagnosing photodermatoses in skin of color can be difficult. When compared to lighter skin, the typical indications may be less noticeable, which might result in missing or delayed diagnoses. Hence, a complete patient history is essential, and physicians need to be vigilant for more subtle clinical indicators. An awareness and understanding of these distinct clinical features is necessary for effective care in order to provide a tailored approach to diagnosis, management, and prevention.

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Impact of melanin on DNA photodamage and vitamin D synthesis

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The quantity and quality of constitutive epidermal melanin determine skin colour and responses to solar ultraviolet radiation (UVR). Epidemiology shows that melanin protects against UVR-induced skin cancer, caused mainly by DNA photodamage, especially the cyclobutane pyrimidine dimer (CPD). Synthesis of vitamin D in the epidermis is the only established benefit of solar UVR exposure, particularly UVB (~295-315nm) radiation. People with higher levels of constitutive melanin have poorer vitamin D status compared with less melanised skin types at given latitude ranges. It is widely assumed that melanin inhibits DNA damage as well as vitamin D synthesis, but we lack quantitative data. One approach to assess the impact of melanin is to compare extreme skin phototypes such as Fitzpatrick skin types II with VI. Such studies show that melanin is highly effective at inhibiting CPD, especially in the basal layer that has a much higher concentration of melanin than the suprabasal epidermis [1]. It was estimated that melanin afforded a protection factor of ~60 in the basal layer that contains keratinocyte stem cells and melanocytes. This level of protection may explain differences in skin cancer incidence in black and white skins. However, comparable studies show that melanin has a very modest inhibitory effect (factor <1.5) on vitamin D synthesis [2]. The most likely reason for large quantitative differences between protection of CPD and inhibition of vitamin D synthesis is the spatial relationship between melanin and photobiological target. In the case of CPD, there is a high concentration of melanin directly above the nuclei of the basal layer cells. In the case of vitamin D, there is sufficient precursor (7-dehydrocholesterol) above the highly melanised basal layer to allow vitamin D synthesis. In summary, it is important to recognise that the effect of melanin depends on endpoint and that blanket statements on melanin are not useful.

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Photoprotection: Addressing challenges for skin of color

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Photo protection has historically been targeted towards individuals with lighter skin tones due to increased risk of sunburn reactions and skin cancer. However, in recent years, the effects of Ultraviolet Radiation and Visible Light have been shown to impact darker skinned individuals as well, contributing to the development and exacerbation of pigmentary disorders in addition to photoaging. However, there are several challenges to photoprotection in skin of color (SOC) populations including issues surrounding the classification of skin phototypes, misconceptions on the need for photoprotection in SOC, and cosmetic unacceptability of traditional sunscreens. However, measures are being taken to mitigate these challenges and promote photoprotection strategies that are acceptable to those with SOC, including a shift to more individualized photo protection.

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Synergistic Strategies for Photodynamic Therapy: Harnessing Block Copolymer Nanosytems for Enhanced both Drug Delivery and Photosensitizers Activity

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Photodynamic therapy (PDT) has emerged as a versatile therapeutic approach in medical fields like ophthalmology, oncology, and dermatology, leveraging the localized production of reactive oxygen species induced by a photosensitizer's interaction with oxygen in biological tissues. Nanocarriers, particularly block copolymer micelles, have been extensively employed to deliver hydrophobic photosensitizers, enhancing their pharmacokinetics and bio-distribution. However, the mechanisms underlying nanocarrier internalization remain unclear, prompting focused studies on copolymer micelle-membrane interactions.

A recent study investigated the efficacy of two copolymer micelles, poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-PCL) and poly(ethylene oxide)-*block*-polystyrene (PEO-PS), in delivering the photosensitizer Pheophorbide a (Pheo). While both micelles exhibited similar capabilities in delivering Pheo to model membranes, PEO-PCL demonstrated higher cellular uptake. Interestingly, this increased uptake did not correspond to improved PDT outcomes, suggesting subtle differences in micellar behavior affecting Pheo delivery.

Furthermore, encapsulating Pheo within PEO-PCL micelles significantly enhanced its cellular responses in human colorectal tumor cells, eliciting notable morphological, mitochondrial, and metabolic alterations. This comprehensive investigation

underscores the pivotal role of the photosensitizer's delivery system in shaping therapeutic outcomes, emphasizing the need to consider both components in PDT studies.

Moreover, advancements in understanding photodynamic therapeutic efficiency have extended to the characterization of polymeric self-assemblies, evaluating their purity and morphological diversity. Controlled mixtures of micelles and vesicles demonstrated synergistic effects, surpassing monomorphous systems in PDT efficiency, highlighting the potential superiority of polymorphous vectors in therapeutic applications. Finally, the integration of terahertz spectroscopy into PDT research offers real-time insights into plasma membrane alterations during treatment. By examining early events in membrane permeabilization, this technique provides sensitive, time-resolved information crucial for understanding PDT mechanisms and optimizing therapeutic protocols.

Together, these studies contribute to a deeper understanding of PDT mechanisms, nanocarrier dynamics, and cellular responses, paving the way for improved therapeutic strategies and clinical applications.

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Can phthalocyanines be successful photosensitizers in vascular-targeted photodynamic therapy? Evidence from photochemical studies and biological evaluation on hiPSC-derived organoids and rodent models

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Photodynamic therapy is based on the effective excitation of a photosensitizer at the appropriate time after its administration (drug-to-light-interval, DLI) to produce ROS.[1] Our research interests have focused on the photochemical properties of nanoformulated photosensitizers from the groups of bacteriochlorins[2] and phthalocyanines[3] and their application either in vascular targeted PDT (V-PDT, DLI=15 min) or cellular targeted PDT (C-PDT, DLI=24h or 72 h).

Our newly synthesized phthalocyanines are effective generators of reactive oxygen species (ROS). $PtSO_2tBu$ demonstrated an outstanding ability to generate singlet oxygen ($\Phi_\triangle$ = 0.87-0.99), while $ZnSO_2tBu$ in addition to $^1O_2$ ($\Phi_\triangle$ = 0.45-0.48) generated efficiently other reactive oxygen species, in particular ·OH. To facilitate their biological administration, a water-dispersible formulation of these phthalocyanines was developed using triblock copolymers to improve their delivery to cancer cells and tissues. The results showed a significant increase in cellular uptake when the phthalocyanines were incorporated into the customizable polymeric micelles. Moreover, the improved distribution in the body and photodynamic efficacy of the encapsulated phthalocyanines were investigated in hiPSC-delivered organoids and BALB/c mice bearing CT26 tumors. Vascular-targeted photodynamic therapy (V-PDT), led to complete tumor eradication in 84% for $ZnSO_2tBu$ and 100% for $PtSO_2tBu$ treated mice, and no recurrence has so far been observed for up to two months after treatment. In the case of $PtSO_2tBu$, the effect was significantly stronger, offering a wider range of light doses suitable for achieving effective PDT.

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Inducing Immunogenic Cancer Cell Death through Oxygen-Economized Photodynamic Therapy with Nitric Oxide-Releasing Photosensitizers

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Photodynamic therapy (PDT) utilizes reactive oxygen species (ROS) for eradication of cancer cells. Its effectiveness is governed by the oxygen content, which is scarce in the hypoxic tumor microenvironment. We report herein two zinc(II) phthalocyanines substituted with two or four nitric oxide (NO)-releasing moieties, namely **ZnPc-2NO** and **ZnPc-4NO**, which can suppress the mitochondrial respiration, thereby sparing more intracellular oxygen for PDT. Using HT29 human colorectal adenocarcinoma cells and A549 human lung carcinoma cells, we have demonstrated that both conjugates release NO upon interaction with the intracellular glutathione, which can reduce the cellular oxygen consumption rate and adenosine triphosphate generation and alter the mitochondrial membrane potential. They can also relieve the hypoxic status of cancer cells and decrease the expression of hypoxia-inducible factor protein HIF-1α. Upon light irradiation, both conjugates can generate ROS and induce cytotoxicity even under a hypoxic condition, overcoming the oxygen-dependent nature of PDT. Interestingly, the photodynamic action of **ZnPc-**

2NO elicits the release of damage-associated molecular patterns, inducing the maturation of dendritic cells and triggering an antitumor immune response. The immunogenic cell death caused by this oxygen-economized PDT has been demonstrated through a series of in vitro and in vivo experiments.

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Stroma- and immune-modulating photosensitizing systems: antibody conjugates, lipid nanoparticles, and everything in between

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Our team's work focuses heavily on the impact of photodynamic action on the stroma of solid tumors. More recently, we have also began to focus on the stroma-immune axis to enable more favorable responses to immune checkpoint therapy in pancreatic cancer and head and neck cancer. Our team is achieving this by constructing various iterations of antibody conjugates, lipid nanoparticles (and everything in between) with the primary focus of disrupting the immunosuppressive tumor microenvironment. This talk will describe our team's efforts to re-engineering photosensitizing systems in order to modulate their photochemistry, and in turn, favor stroma-disrupting and immune enhancing treatment protocols for pancreatic cancer and head and neck cancer.

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Formulating and delivering phthalocyanines to cancer cells and tissues

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While synthesizing water-soluble amphiphilic phthalocyanines for photodynamic therapy applications is achieved with different substitution patterns (1), it is also of interest to use more hydrophobic derivatives, which then need to be formulated or conjugated to be delivered to cancer cells and tissues. Over the years we have developed several strategies, such as the grafting of photosensitizing phthalocyanines onto biocompatible polymers such as polycaprolactone (2) or poly-L-glutamic acid (3), or their incorporation into silsesquioxane nanoparticles (4). The encapsulation of non-functionalized phthalocyanines into Pluronic-based micelles (5) or other micellar formulations (6) has also been achieved. The design of phthalocyanines' chemical structures depending the conjugation / encapsulation / formulation will be presented and the different advantages and drawbacks will be discussed.

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What is the phyB photobody made of?

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Phytochrome B (phyB) is a plant red light photoreceptor that regulates various developmental processes including germination, seedling photomorphogenesis and shade avoidance response. Upon the absorption of red light, phyB enters the nucleus and forms a membraneless organelle called photobody, suggested to be a site for transcription, protein sequestration and protein degradation. However, which proteins constitute the photobody is still elusive. We report the isolation and the identification of phyB photobody components. We isolated the photobody by adopting the fluorescence-activated particle sorting and analyzed the isolated photobody components with the liquid chromatography-tandem mass spectrometry. Our analysis shows that a phyB photobody is made of about 1,500 phyB dimers and other proteins that could be classified into two groups based on their requirement of other proteins to localize to the photobody in protoplasts. The first group includes proteins that directly interact with phyB and readily localize to the phyB photobody when transiently expressed in protoplasts, while the second group includes proteins that require the co-expression of the first group to localize to the photobody. Together, our results support that phyB photobodies include not only phyB and its primary interacting proteins but also its secondary interacting proteins.

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Function of photobodies in phytochrome signaling in plants

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Phytochrome B (phyB) is a red/far-red photoreceptor and thermosensor that regulates all aspects of plant development and growth by controlling the expression of hundreds of light- and temperature-responsive genes. PhyB reprograms the nuclear genome by regulating the stability and activity of a group of basic helix-loop-helix transcription factors called PHYTOCHROME-INTERACTING FACTORs (PIFs). At the cellular level, one of the earliest light responses is the translocation of photoactivated phyB from the cytoplasm into the nucleus and the subsequent condensation of phyB into discrete subnuclear organelles named photobodies (PBs). It was proposed that PB formation is driven by the liquid-liquid phase separation (LLPS) of active phyB. Accumulating genetic and biochemical evidence suggests that PB formation correlates with phyB signaling and light responses. For example, PBs contain many phyB signaling components including PIFs, and PBs have been associated with PIF3 degradation and PIF7 sequestration. However, because phyB and other PB components are diffusible between PBs and the surrounding nucleoplasm, the main challenge of studying PB functions has been the difficulty in dissecting the function of phyB signaling between the PB and nucleoplasmic compartments. As a result, the current data still cannot unequivocally assign a function exclusively to the PB compartment. I will discuss our recent progress in defining the function of PBs in phyB signaling and the regulation of PIFs.

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Role of CRY2 condensates in controlling light-responsive gene expression in *Arabidopsis*

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Cryptochromes (CRYs) are blue light receptors that control many aspects of plant growth and development through regulating gene expressions. In 2000, a study reported an interesting reaction that *Arabidopsis* CRY2-RFP (Red Fluorescent Protein) fusion proteins formed nuclear speckles in response to blue light. Similar observation was later made with the endogenous *Arabidopsis* CRY2 proteins, indicating that light-induced aggregation is a native photo-response of CRY2 photoreceptors. We recently demonstrated that these CRY2 nuclear speckles were liquid protein condensates in nature that formed via light-dependent liquid-liquid phase separation (LLPS). Accumulating evidence over the past few years reveals that CRY2 condensates may play important roles in mediating photo-regulation of gene expression at multiple levels, by co-condensing CRY2-interacting proteins. We previously showed that CRY2 interacted with the METTL3/14-type m^6A RNA methyltransferase complex (or m^6A writer complex) *in vivo* in a blue light-independent manner but assembled it into CRY2 condensates in response to blue light. The concentrating of m^6A writer complexes in CRY2 condensates might facilitate m^6A RNA methylation of transcripts encoding key circadian clock oscillator genes (such as *CCA1*) thus modulate circadian clock activities. In this talk, I will present more of our findings towards understanding how CRY2 condensates contribute to the light-dependent regulation of gene expression at the transcriptional and co-transcriptional levels eventually resulting in light-responsive growth of plants.

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Translation control by the cytosolic biomolecular condensate P-bodies optimizes early seedling developments in Arabidopsis

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Dark-grown Arabidopsis seedlings can sequester specific mRNAs that encode proteins for photomorphogenesis in processing bodies (p-bodies) to attenuate their translation before exposure to light (Jang et al. 2019). However, how this selected sequestration is achieved and its biological impacts remain to be investigated. DCP5 (Decapping 5) is one of the p-body components with an RNA binding motif; therefore, it has the potential to bind and recruit mRNAs into p-bodies. We adopted infrared-CLIP (irCLIP) and RNA-seq to reveal direct target mRNAs of DCP5. Among the candidate mRNAs include *CHL27* and *TCP14*. *CHL27*encodes a protochlorophyllide biosynthetic enzyme magnesium-protoporphyrin IX monomethyl ester cyclase, and TCP14 (TEOSINTE BRANCHED, CYCLOIDEA AND PCF 14) is a transcription factor functioning in etioplast to chloroplast transition and in activating *ELIP1 (Early light-induced protein 1)* and *ELIP2* to promote cotyledon greening efficiency. We confirmed DCP5 associates with *CHL27* and *TCP14* mRNAs in p-bodies via specific binding sites. Our results also showed that, in dark-grown seedlings, DCP5 functions to recruit and stall the translation of *CHL27* and *TCP14* mRNAs to achieve a balanced chlorophyll accumulation and chloroplast development when transitioning to the light environment. This sequestration ensures a timely and precise switch from skotomorphogenesis to photomorphogenesis development in Arabidopsis seedlings.

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Circadian Rhythm Regulation by Iron Deficiency and Chloroplast Signaling in *Arabidopsis thaliana*

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The homeostasis of iron (Fe) in plants is crucial for optimal growth and development while preventing oxidative stress. However, about 30% of arable land suffers from Fe deficiency due to calcareous soil conditions. In Arabidopsis, Fe deficiency leads to retarded growth, chloroplast dysfunction, and delayed flowering. This study investigates the interplay between Fe availability and the circadian clock in growth and development. Under Fe-deficient conditions, Arabidopsis exhibits a longer circadian period, potentially mediated by plastid-to-nucleus retrograde signaling. Mutant analysis reveals that central oscillator genes are essential for the Fe deficiency-induced lengthening of the circadian period. Establishing an EMS mutant database identified mutants with altered Fe-deficient circadian responses, highlighting the role of Fe in circadian regulation. The study sheds light on the mechanism of circadian rhythm lengthening via the chloroplast retrograde signaling pathway, offering insights into plant responses to environmental stimuli.

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From color-tuning to optogenetics: relationship between red-light absorption and fluorescence intensity in an archaerhodopsin model.

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Recent computational studies have revealed the critical impact of the electronic structure of the excited state species generated during the light-induced double bond isomerization of the chromophore of rhodopsin pigments. While a charge transfer structure dominates the function of visual rhodopsins (1), a diradical structure appears to control the fluorescence emission in NeoRhodopsin (3,4) and in a set of archaearhodopsin optogenetic reporters (2). By using a novel and unconventional type of quantum chemical modeling, we show that the stability of such a diradical must be inversely proportional to the absorption wavelength and that, most importantly, both properties can be regulated by the position and “delocalization” of the counter-ion of the protonated retinal chromophore.

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Multiscale conformational dynamics in proteins and DNA probed by time-resolved circular dichroism from femtoseconds to milliseconds

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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 biological molecules over a wide range of time scales.

Despite recent technological advances, time-resolved CD experiments at the femto-picosecond time scale remain challenging, due to their very weak signals prone to artifacts [1]. In recent years, we have designed several setups to measure the light-induced CD changes of various chiral compounds over a time window ranging from a few hundred femtoseconds to a few seconds [2,3].

In this presentation, I will discuss the principle, advantages and drawbacks of these setups. I will illustrate their applications for studying the photoinduced conformational changes of proteins and short G-quadruplex DNA structures [4,5].

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Light activation mechanism of Orange Carotenoid Protein resolved by femtosecond stimulated Raman Spectroscopy

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The Orange Carotenoid Protein (OCP) is a distinctive water-soluble keto-carotenoid binding protein primarily found in cyanobacteria, crucial for non-photochemical quenching—a protective mechanism dissipating excess absorbed light energy as

heat, shielding photosynthetic organisms from photodamage. Upon photon absorption, hydrogen bonds within the carotenoid-protein complex break, transitioning OCP from its orange to red-absorbing form, enabling binding to Phycobilisome. The process's enigmatic nature stems from its exceptionally low quantum yield, likely a result of evolutionary adaptation to high light conditions. Employing steady-state and time-resolved spectroscopy, notably fs stimulated Raman spectroscopy, we systematically investigated the vibrational characteristics of optically excited echinenone in various solvents and OCP in both red and orange states. Our innovative technique, employing synchronized fs amplifiers and controlled pump pulse displacement, facilitated recording of OCP photoactivation dynamics from 70 fs to 10 ms, spanning a staggering 12 orders of temporal range in a single experiment. Our findings unveil a unique multiphoton activation pathway, suggesting OCP's light intensity sensing capability. While early photoproducts tend to revert to their original configuration, the probability is significantly altered upon receiving additional photons within a specific timeframe. Our coherent Raman experiment sheds light on the carotenoid's heat dissipation pathways within OCP, revealing distinct vibration bond excitation profiles compared to those observed in solution. This evidence supports the notion that OCP has evolved to control energy dissipation in carotenoids, directing it into the protein through controlled pathways, indicative of evolutionary adaptation.

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Spectroscopic study on the photoreaction dynamics of ion-transporting microbial rhodopsins

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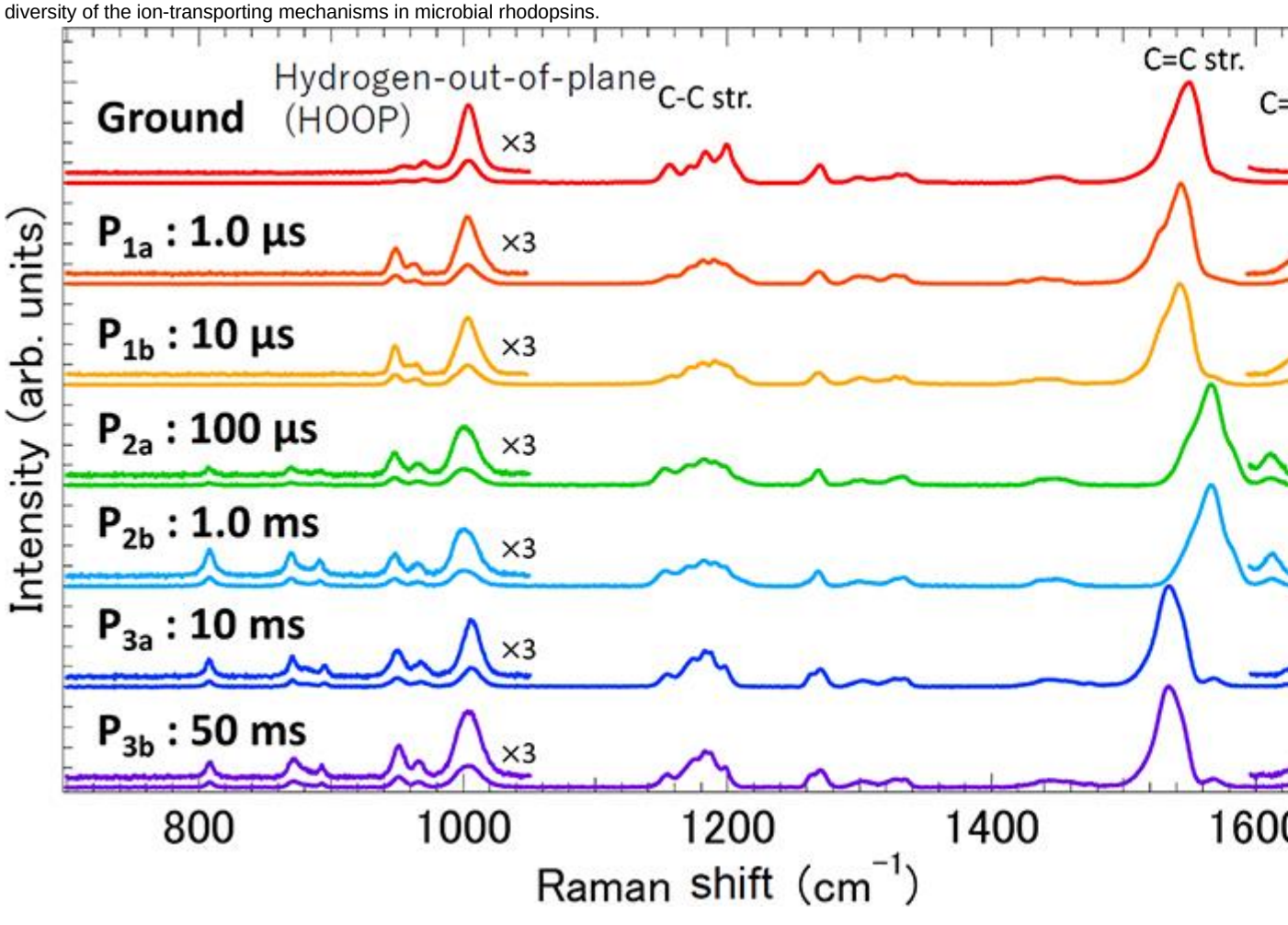
Channelrhodopsins (ChR) are light-gated ion channels that passively transport various cations and anions in a light-dependent manner. While these proteins are widely used in optogenetics to optically control the neural activity *in vivo*, their channel gating mechanism has not been clarified yet. Recent quantum mechanics/molecular mechanics (QM/MM) calculation and time-resolved X-ray crystallography observed that the structure of the retinal chromophore in C1C2, which is a chimeric ChR constructed by combining the amino acid sequences of *Cr*ChR1 and *Cr*ChR2 found in *Chlamyidomonas reinhardtii*, is highly distorted in the pre-open state of the protein [1,2]. This structural change in the retinal chromophore is considered to probably facilitate the subsequent channel opening. Here, we studied the photoreaction dynamics of C1C2 by transient absorption spectroscopy, laser patch-clamp, and time-resolved resonance Raman spectroscopy. As previous studies suggested, an increase in the hydrogen-out-of-plane modes indicating the larger twisting of the retinal chromophore compared to the ground state was observed in the photointermediate states. This twisting reached maximum in the P_{2b} state, which was identified as the full-open state through the laser patch-clamp measurement. The retinal twisting is relaxed in the subsequent P_{3b} state, and this process is rate-limited by a H^+ transfer from the protein moiety to the Schiff-base linkage of the retinal chromophore. Our result indicates that the twisting of the polyene chain of the retinal chromophore, which is not observed in other types of microbial rhodopsins, induces the opening of the channel pore in C1C2 [3]. In this presentation, I will present the results of other ChRs and ion pumping rhodopsins and the diversity of the ion-transporting mechanisms in microbial rhodopsins.



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To twist or not to twist: photoisomerization bottlenecks in negative reversibly photo switchable fluorescent proteins

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Natural photoactive proteins serve both as inspiration and as templates for efforts to design new proteins with photofunctions beyond the biological context of the template. Inevitably, changing the amino acid composition will modulate properties, but there is no systematic understanding of how the desired excited-state control can be achieved nor how modifiable a given template can be. One example is reversibly photoswitchable fluorescent proteins that are key actors in enabling bio imaging beyond the optical diffraction limit. Yet, their photoisomerization quantum yields are consistently low, hampering their use in active control applications. Understanding the origin of these limitations could facilitate breaking free of the photo functional optimum of the template. In this talk, I will discuss our recent efforts to unravel photoisomerization bottlenecks in negative reversibly photo switchable fluorescent proteins using an arsenal of computational tools.

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How the protein cage controls the photoswitching mechanism of reversibly photoswitchable fluorescent proteins.

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Recently, reversibly photoswitchable fluorescent proteins (RSFPs) have been widely applied in super-resolved fluorescence microscopy, such as reversible saturable optical fluorescence transition (RESOLFT), a super-resolved microscopy technique that allows for a significant reduction in the illumination intensities and in photobleaching. Even though photo-physical parameters (switching, fluorescence quantum yields…) are linked to the resolution and image acquisition speed, the switching mechanism that controls these parameters is still a matter of debate. The most studied RSFP is Dronpa, a negative RSFP from Anthozoa (e.g. corals). Using a combination of time-resolved crystallography and transient absorption spectroscopy we studied the mechanism of off-to-on and on-to-off photoswitching in WT and different mutants of rsEGFP2 (e.g. jellyfish), a common protein used in super-resolved microscopy. We clarified the order of off-to-on photoswitching events, i.e. chromophore isomerization in the picosecond time scale with the formation of a twisted chromophore[1], and different ground-state steps with protein conformational changes and a deprotonation on the microsecond timescale[2, 3]. We will then discuss here our recent results for on-to-off switching and how the protein cage controls meaningful parameters for WT rsEGFP2 and mutants in comparison to other RSFPs: fluroescence and switching quantum yield [4].

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Engineering photoreceptors into optogenetic tools for the control and understanding of cellular processes in microbial, animal and plant systems

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Abstract content TBC

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Optogenetic stimulation of projection neurons – sublime but not physiological

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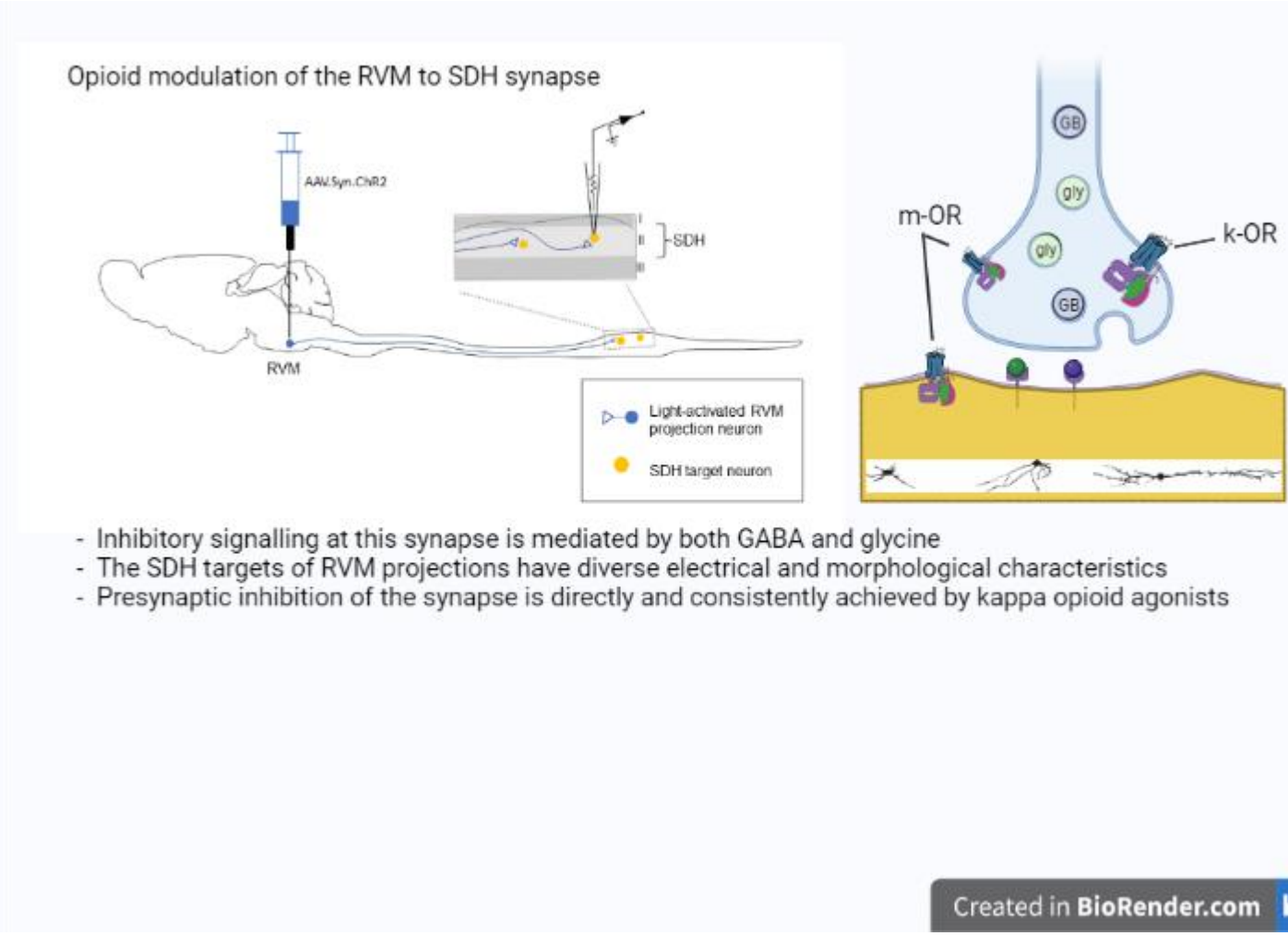
Advances in neurosciences are largely driven by technology. The combination of optogenetics with modern genetic and fiberoptics tools have allowed researchers to investigate brain function, and the cells and circuits governing it, in unprecedented detail. My group uses optogenetics and other tools to investigate pain circuits: how they signal and are modulated, what individual cell groups do within the circuit, how complex and dynamic responses to pain are organised, and what alterations in the system result in chronic pain states.

To improve knowledge about a key descending pain modulatory system, we used optogenetics to selectively stimulate one part of this pathway in isolation. We focused on the final synapse made between projection neurons located in the brainstem and neurons in the dorsal horn of the spinal cord, where nociceptive signals first enter the central nervous system. We found that brainstem inputs release the neurotransmitter glycine along with GABA and signal through a multitude of different neuronal types throughout the spinal dorsal horn. Then we showed that activation of kappa-opioid receptors reliably inhibits transmitter release from these descending inputs. This study improves our mechanistic understanding of how descending pain pathways and opioids control pain sensation and enhances our ability to design and develop safe and effective new analgesics. In addition to this example of how optogenetic tools are valuable in neuroscience research, I will also framework some of the ongoing experimental challengers using optogenetic tools to investigate neuronal function.



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Photochemical internalization (PCI). From microscopy to clinic.

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The short lifetime and thereby the diffusion length of singlet oxygen as well as the correlation between photosensitizer (PS) fluorescence and treatment effects made it attractive in the 80-ties to analyze the intracellular localization of PSs to reveal the initial intracellular hit in PDT. Some PSs were found intracellularly in fluorescing granules that was found to be lysosomes and endosomes. Such vesicles were rupture upon exposure of the cells to light and the PS relocated to other compartments in the cells. About 40 different hydrolytic enzymes are found in late endosomes and lysosomes. Release of lysosomal hydrolases into cytosol has been documented to be a cytotoxic event. This suicide sac hypothesis suggests that released hydrolases lead to impairment of the cells and the cells subsequently die. However, it was found that a large fraction of the vesicles containing PSs could be ruptured without inducing substantial cytotoxicity. The scientific basis for this surprising observation as well as the potential utilization of the photochemical rupture of endosomes and lysosomes for intracellular delivery of various therapeutics, named photochemical internalization (PCI), will be discussed. The PCI technology may be utilized for delivery of all type of molecules accumulating in endocytic vesicles as documented for peptides, protein, oligonucleotides (siRNA and PNA), genes

delived by polycationic vectors, adenoviral and adenoassociated vector, for improved antigen presentation for cancer vaccination as well as some small molecular drugs such as bleomycin. Outcome of some clinical trials will also be presented. During the last 20-30 years macromolecular therapeutics have become increasingly attractive for treatment of cancer due to their improved specificity and reduced side effects. Intracellular delivery of macromolecular therapeutics has however so far showed only limited success, a limitation that may be circumvented by PCI. The historic and scientific basis for PCI will be presented.

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Where do microbial rhodopsins come from? What are they? Where are they going?

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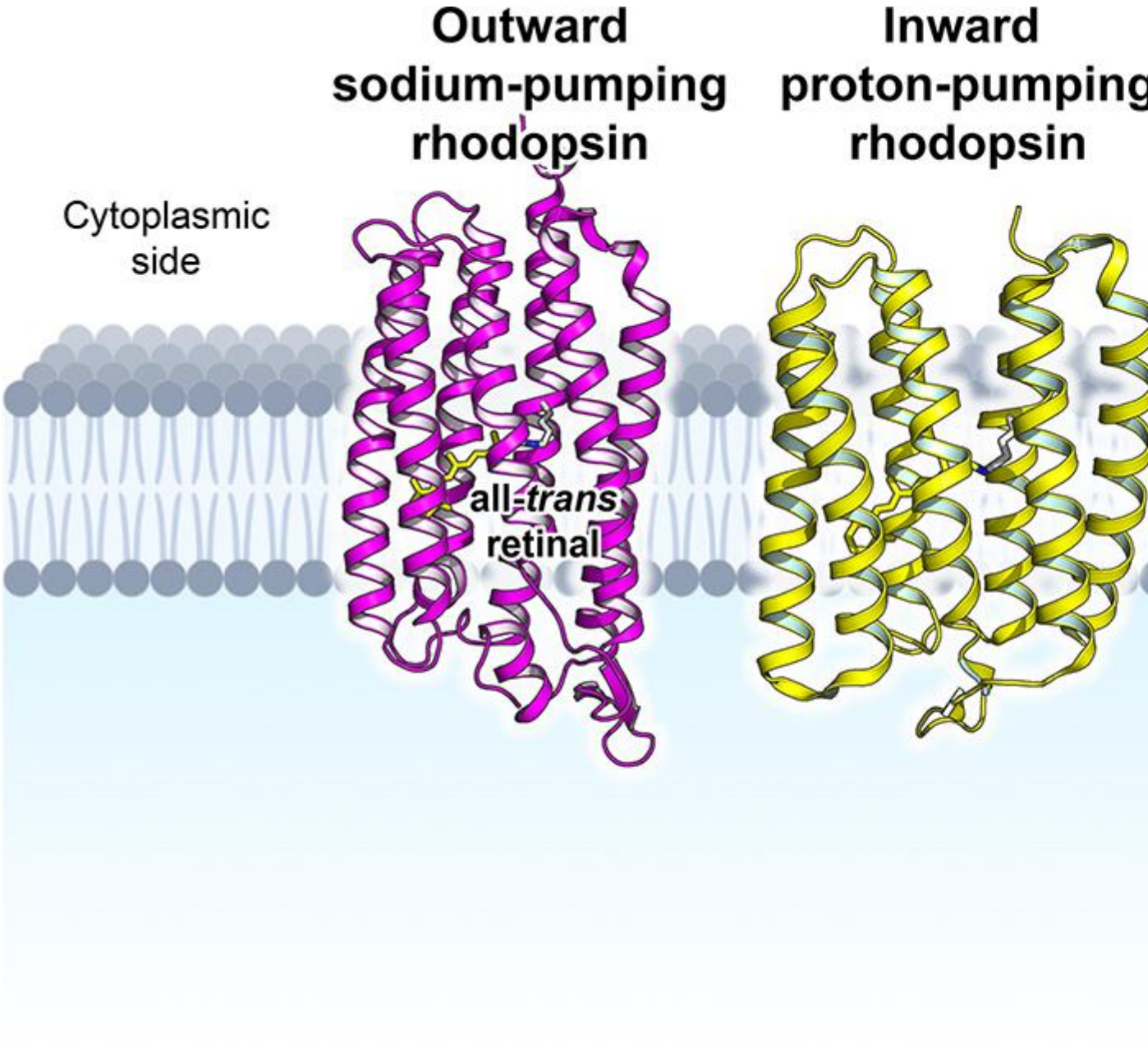
Microbial rhodopsins are photoreceptive heptahelical membrane proteins that use an all-*trans*-retinal chromophore covalently bound to a conserved lysine residue in the seventh transmembrane helix through a protonated Schiff-base linkage. Upon Light absorption, the retinal undergoes all-*trans*-to-13-*cis* photoisomerization, initiating a photocyclic reaction accompanying a series of conformational change of the protein, thereby eliciting the biological function. While most microbial rhodopsins act as outward light-driven H^+ pump, we discovered a new outward Na^+-pumping rhodopsin, KR2, in the genome of the marine flavobacterium, *Krokinobacter eikastus* [1]. The structural and spectroscopic analyses revealed that intramolecular proton transfers are coupled with the Na^+ transport through transiently neutralizing the positive charge of the protonated retinal Schiff base [2-3]. Through a comparison of amino acid sequences between KR2 and outward H^+ pumps, we found that three residues in the third transmembrane helix play an crucial role in determining the substrate ion species and transport direction; these residues are referred to as motif residues. By focusing microbial rhodopsin genes with motif residues different from known groups, we identified two types of inward H^+-pumping microbial rhodopsins [4-6]. X-ray structural analysis of these inward H^+ pumps indicated that a single, and fewer in number compared to outward H^+ pumps, counterion and a unique acidic residue on the cytoplasmic side facilitate the transport of H^+ in the opposite direction to that of outward H^+ pumps [7]. In 2018, heliorhodopsin, which forms a new family distinct from canonical microbial rhodopsins and has an inverted protein orientation with N- and C-termini facing the cytoplasmic and extracellular sides, respectively, was discovered through functional metagenomics [8]. Structural analysis showed a large fenestration between the fourth and fifth helices in heliorhodopsin, and biochemical assay suggested exogenous all-*trans*-retinal binds to the binding pocket in the protein through this fenestration [9]. Further genomic, metagenomic, and functional metagenomic surveys identified many new functional microbial rhodopsin groups [10,11]. These findings of new types of microbial rhodopsins are changing our traditional view of this protein family, which has been established over the past century. I will present our studies on these new microbial rhodopsins and offer perspectives on future research.



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Usefulness of the SmartPDT® digital medical device to optimise the effectiveness and safety of natural daylight PDT (NDL-PDT): a clinical study in Spain

Yolanda Gilaberte[1], Tamara Gracia-Cazaña[1], Alba Navarro-Bielsa[1], Oriol Yelamos[2], Manuel Almenara-Blaso[1], Ana J Garcia-Malinis[3], María Gimenez-Sanmartin[1], Rowan C Temple[4], Marco Morelli[4]

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4. SiHealth Ltd, Harwell Campus, Oxfordshire, United Kingdom

Natural Daylight Photodynamic Therapy (NDL-PDT) is an efficacious treatment of actinic keratosis (AK). However, the use of daylight introduces uncontrolled variability that may influence the effectiveness, such as time of year, cloudiness, sunscreen application and patient behaviour [1]. An innovative satellite-based solution (SmartPDT®) is the first scientifically validated digital

medical device (CE-marked Class 1) solving this [2]. The dermatologist can accurately plan and then monitor in real-time the effective (PpIX-effective) and safe (erythemal) solar radiation doses.

An observational, multicentre, prospective study of clinical practice took place in Spain from June 2022 to October 2023. Clinical teams used the SmartPDT® web-portal for monitoring either a hospital-based NDL-PDT or a home-based NDL-PDT performed by the patient using its related mobile app. Follow-up clinical evaluation was performed at 3 months.

Thirty patients were included, 5 females and 32 males, with ages ranging from 51 to 87 years old. All NDL-PDT sessions were performed according to the current clinically-accepted therapy protocol [3], so exposing patients for exactly 2 hours to sunlight independently from weather conditions. AK severity (AKASI score) was assessed before and 3 months after treatment, considering scalp, forehead, left face and right face separately.

For all body sites considered, PpIX-effective solar doses ranged from 2.98 J_{eff}/cm^2 to 23.8 J_{eff}/cm^2, while erythemal doses ranged from 1.9 J_{eff}/m^2 to 61.84 J_{eff}/m^2. Air temperature ranged from 7.3 ˚C to 39.58 ˚C. A preliminary analysis on the correlation between AKASI variation (i.e. treatment effectiveness) and monitored environmental variables was conducted and we will present its results.

This study demonstrates that a satellite-based digital system can help clinicians to optimise the overall management and effectiveness of NDL-PDT, planning and monitoring the environmental variables affecting its clinical outcomes and safety. This can help to provide a more effective and comfortable treatment with higher therapy adherence.

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Long-lasting and safe photoprotection using a skin-bioadhesive technology: a proof of concept with a novel M10 skin-bioadhesive UVA filter - SPONSORED BY: SKINOSIVE

Juliette Bertrand[1], Lise Ainié[1], Nina Séjourné[1], Amélie Dupont[1], Mehdi Makrerougras[1], Laura Studenski[1], Maora Kessaci[1], Isabelle Rault[1], Benoit Canolle[1]

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Protection against solar UV radiation is a global public health need due to the increasing incidence of skin cancer over the last decades. Innovating in sunscreen is a challenging research and technology effort. Health authorities and consumers support the use of safe sunscreens that are highly protective, without causing adverse environmental effects. Organic UV filters, while providing cosmetic advantages, require frequent re-application, may penetrate the skin, and their release in the aquatic environment can have a significant impact on the marine ecosystem with special impact on coral. Based on breakthrough skin-bioadhesive technology, we have developed new organic UV filters that bind to the stratum corneum.

Skin-bioadhesive technology is based on the generation of innovative molecules with a core of known commercial UV filters with a bioadhesive group that binds to thiols at the surface of the skin. Among new UV filters with this technology, the UVA DHHB (diethylamino hydroxybenzoyl hexyl benzoate)-derived bioadhesive UV filter, M10, is the most advanced compound and was formulated for skin explants and human clinical tests. The efficacy and safety of M10 were evaluated *in vitro* and *ex vivo* using UV spectrum analysis, skin autofluorescence, UVA imaging, diffusion cell permeation and Raman confocal spectroscopy. The persistence of photoprotection over time was analyzed *in vivo* in a clinical study using UVA imaging facilities on human volunteers' arms (N=15).

In vitro and *ex vivo*, comparison of M10 to DHHB formulated at the same molar concentration in the same chassis, demonstrated superiority of the bioadhesive M10 with respect to its efficacy, persistence and resistance to washes. Most importantly, the long-lasting photoprotection of M10 was observed *in vivo* in a clinical study as a human proof of concept of the technology. M10 showed a good tolerance, resistance to rubbing, reduced transversal skin diffusion and a persistence of photoprotection: M10 was significantly 47% more protective than DHHB, with 84% persistence 6 hours after application. The consequent reduction in the penetration of bioadhesive UV filters over the stratum corneum was confirmed by Raman confocal spectroscopy on human skin explants.

Studies with a novel UVA M10 UV filter demonstrated that skin bioadhesive technology can bind organic UV filters to the skin surface and the chemically modified UV filter persists in the stratum corneum with no accumulation in deeper skin layers, while preserving the active protective spectrum. We clinically observed a long-lasting photoprotective effect over 6 hours, substantially longer than the recommendation to reapply sunscreen every two hours. In addition to M10, other skin-bioadhesive UVA and UVB filters are being developed. The development of safe and long-lasting skin-bioadhesive UV filters could be a major advance in photoprotection research that the industry has been seeking over the past 20 years.

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Personalized photoprotection

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The cutaneous photobiologic effects of UVB and UVA are well known, which include erythema, tanning, and with chronic exposure, photocarcinogenesis. Visible light is now known also to have photobiologic effects on the skin, specifically intense and persistent pigmentation most noticeable in dark skinned individuals. This is of particular clinical relevance as pigmentary

alterations on the skin such as post-inflammatory hyperpigmentation and melasma are more commonly observed in dark skinned individuals.

Comprehensive photoprotection consists of seeking shade, wearing photoprotective clothing, wide brimmed hat and sunglasses, and applying sunscreen to otherwise exposed sites. With diversity of skin phototypes of individuals globally, the concept of *personalized photoprotection* in terms of sunscreen usage has become an important component of photoprotection education of the public. Fair skinned individuals have high risk of sunburn and skin cancer development. Therefore, high SPF (50+) sunscreens with UVA-PF of >1/3 of labelled SPF should be recommended. Individuals with dark skinned have lower risk of sunburn or skin cancer, however, they are at a high risk to develop UV- and visible light-induced hyperpigmentation. SPF30+ with good UVA-PF (>2/3 labelled SPF) should be recommended. In addition, sunscreens that contain agents that block visible light (tinted, also known as colored sunscreens, or sunscreens that contain new filters that absorb in the visible light range) should also be recommended for these individuals.

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UV-exposure shapes melanoma biology & response to treatment

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Cutaneous melanoma is the most serious type of skin cancer. Its development is closely linked to exposure to solar exposure namely ultraviolet (UV). It is now well established that UV radiation increases the risk of development of melanoma and melanoma incidence correlates with the intensity of the sun exposure together with the skin's photo-type. In white population, the incidence generally increases with decreasing latitude, the highest being recorded in Australia.

UV radiation shapes the biology of the skin cells, damaging DNA, with the production of typical C-to-T transition, inducing marks at the epigenome, modulating gene expression program and the cellular response. The deep characterization of these UV-induced modifications is of utmost importance to better understand the biology of those cancer cells and propose adapted therapies.

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Innovative medical app that uses real-time satellite data and AI to optimise sun exposure behaviour

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Public health campaigns advise minimising solar UV radiation (UVR) exposure to prevent skin cancer. However, solar exposure also has health benefits, especially vitamin D synthesis. Thus, effective balancing of risks and benefits of solar UVR would be ideal if personalised recommendations were supported by accurate real-time solar dosimetry.

An innovative CE-marked medical app (Sun4Health®) has been recently developed for this purpose. The app performs real-time whole-body monitoring of both erythemal and vitamin D-effective solar UVR doses using patented technology that combines satellite data and AI-enabled automatic indoor/outdoor positioning [1,2], also incorporating the spectral transmittance and application quantity of the sunscreen applied. This enables personalised recommendations on optimal sun exposure time and sunscreen use with body-site specific recommendations. The app has been evaluated in two clinical studies.

First, a field study in Brazil, was performed to evaluate the app in a beach scenario with high UV index (UVI) [3]. 59 healthy volunteers were randomised into 3 groups, each given a different app providing: (1) UVI only (control), (2) personalised recommendations (Sun4Health®), (3) as (2) but with an additional wearable device that provides body site information (Sun4Health®-3D). Participants were offered sunscreens to use at their discretion. The results show that the app is safe and can modify behaviour to reduce erythema (28% less than control, 33% with 3D version), yet not decreasing vitamin D status.

Secondly, a randomised trial (n=100) in the UK has evaluated the efficacy of the app in real-world everyday conditions [4]. Preliminary results show good efficacy in supporting safe sunlight exposure to achieve personal daily recommended vitamin D synthesis (assuming oral equivalent of 400 IU/day as target).

Overall, clinical studies demonstrate that the app is safe to use and can effectively provide personalised real-time support to users for balancing risks and benefits of solar exposure.

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Photoimmunoconjugate nanoconstructs and their multi-tiered cancer targeting mechanisms

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It is increasingly apparent that optimal cancer therapies will necessitate combined treatments targeting diverse pathways while minimizing adverse effects. The fusion of nanotechnology with photochemistry presents a unique avenue for delivering and activating multiple drugs concurrently, addressing various regions of cancer cells—including the plasma membrane, cytoplasm, and nucleus. PIC-NAL-IRI stands out as a promising approach to overcome the selectivity-uptake trade-off, enhance the efficacy of photoimmunotherapy, and facilitate multi-tiered cancer targeting. Its ability for controlled drug compartmentalization, facile surface modification, and high clinical relevance collectively underscore the significant value of PIC-NAL-IRI for treatment of primary and metastatic cancer.

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Targeted oxidation of HSP90 paralogs induces endoplasmic reticulum stress-mediated immunogenic cell death

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Cancer cells damaged by photooxidation activate the unfolded protein response (UPR) endogenously, a critical process for survival that removes cytotoxic misfolded proteins and maintains protein homeostasis. Heat shock protein 90 (HSP90) paralogs—such as GRP94 in the endoplasmic reticulum (ER), TRAP1 in mitochondria, and HSP90A1 in the cytosol—play essential roles in early UPR stages by recognizing irreversibly damaged misfolded proteins and facilitating their refolding or elimination. HSP90 also maintains the conformation, stability, and function of oncogenic client proteins involved in signal transduction pathways, including proliferation, cell cycle progression, apoptosis, invasion, angiogenesis, and metastasis.[1] However, conventional HSP90 inhibitors like PU-H71 and 17-AAG non-covalently bind to HSP90, showing limited therapeutic efficacy and off-target toxicity in clinical trials.[2]

To address this limitation, we synthesized IrPU, a photosensitizer targeting HSP90, and induced targeted elimination through photooxidation to permanently inhibit its function and reduce cellular levels. IrPU exhibited improved photodynamic therapy (PDT) efficacy under hypoxic conditions, as cellular IrPU uptake increased significantly following elevated levels of GRP94 and TRAP1. Upon photoirradiation, IrPU selectively oxidized and eliminated intracellular HSP90s. Consequently, the reduction in oncogenic client proteins and increased misfolded proteins heightened mitochondrial and ER stress. Post-PDT, activation of the mitochondrial UPR (UPR^{mt}) upregulated proteins involved in mitochondrial folding, antioxidant defenses, and protein quality control, potentially inducing resistance to PDT in UPR-activated cancer cells. Similarly, co-treatment with photosensitizers and HSP90 inhibitors showed a synergistic effect in overcoming PDT resistance.[3] Dysfunction of crucial UPR^{mt}-associated proteins, which are clients of HSP90, resulted in substantial damage to cancer cells during repeated PDT compared to the non-targeted negative control photosensitizer IrCt. Additionally, reduced GRP94 levels increased ER stress, as evidenced by critical dilation of ER morphology, and promoted overexpression and activated translocation of calreticulin and HMGB1, markers of immunogenic cell death. This study promises enhanced efficacy in PDT by overcoming resistance mechanisms and suggesting a potent strategy for future cancer treatments with reduced off-target effects.

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Mechanisms of cell death induced by photo-activated bacteriochlorins that accumulate at the endoplasmic reticulum and Golgi compartments

Lígia C. Gomes-da-Silva[1], **Livia Stenico**[1], **Guido kroemer**[2], **Luis Arnaut**[1]

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Photodynamic therapy (PDT) is an effective treatment for solid tumors, which involves the administration of a photosensitizing agent and subsequent light exposure to generate reactive oxygen species, leading to cell death in the illuminated tissue. The field of cell death has expanded significantly in the last decade, with novel mechanisms and signaling pathways orchestrating multiple cell death pathways being continuously unveiled. While apoptosis is the most well-known and extensively studied regulated cell death mechanism, emerging studies have identified additional regulated cell death pathways such as necroptosis, ferroptosis, pyroptosis, parthanatos, and paraptosis. Some of which have been observed in PDT studies. Furthermore, the immunogenicity of these cell death modalities is under extensive investigation as the field progresses [1].

Over the past few years, we have focused on developing new bacteriochlorins with strong absorption in the NIR, and high phototoxicity and immunostimulatory properties. One such compound, redaporfin (a halogenated sulfonamide bacteriochlorin), has recently completed Phase I/II clinical trials for head and neck cancer (NCT02070432). Another derivative of smaller derivative, LUZ51 (a halogenated sulfonamide bacteriochlorin), has also shown promising results. The mode of action of this family of compounds have been focus of some of our studies. Our results demonstrated that the lipophilicity of these bacteriochlorins favors their accumulation in the endoplasmic reticulum and Golgi compartments, significantly influencing the subsequent biochemical mechanisms of cell death. Photo-activation of these compounds triggers various signs that may indicate the involvement of apoptosis, paraptosis, pyroptosis as well as a non-canonical form of autophagy. These findings highlight the complexity of PDT-mediated cell death and the probable overlap of mechanisms within multiple cell death pathways [2],[3].

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Photodynamic Therapy-Induced Cell Death Based on Targeted Organelles

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The mechanism of cell death in photodynamic therapy (PDT) can vary significantly depending on the targeted organelle due to the specific interactions between the photosensitizer, light, and oxygen within that cellular location. Different cell death mechanism can indeed play a crucial role in overcoming drug resistance, a major challenge in cancer treatment. By utilizing PDT reagent to target specific organelles, it is possible to bypass some of these resistance mechanisms or induce synergistic effect when different target motions are engaged simultaneously. For this reason, my group has developed various PDT reagents targeting endoplasmic reticulum (ER), mitochondria, lysosome, and plasma membranes and investigate their cell death mechanisms depending on the targeted organelle.
In this presentation, I will introduce molecular design strategy aimed at targeting organelles. Our developed photosensitizers will be presented for efficient reactive oxygen species (ROS) generation even in hypoxia conditions, detailing the cell death mechanism with proteomic analyses and phenomenological observations by ROS, and their in-vivo applications.1-3

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Enhanced phototherapeutic efficacy through microbial modulation in cutaneous T-cell lymphoma delays tumour growth and increases survival in the murine EL4 model

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Cutaneous T-cell lymphomas (CTCL), particularly in forms like mycosis fungoides and Sézary syndrome, present unique challenges due to their pathological interactions with microbial elements and resistance to conventional therapies. Our research explores the impact of phototherapeutic interventions combined with targeted microbial modulation on disease progression in CTCL. EL4 T-cell lymphoma cells were intradermally grafted on the back of C57BL/6 mice. Animals were treated with conventional therapeutics such as psoralen + UVA (PUVA) or UVB in the presence or absence of topical antibiotic treatment (neomycin, bacitracin, and polymyxin B sulphate) as an adjuvant. Microbial colonization of the skin was assessed to correlate with disease severity and tumor growth. Triple antibiotic treatment significantly delayed tumour occurrence ($p = 0.026$), which prolonged the survival of the mice ($p = 0.033$). Allocation to phototherapeutic agents PUVA, UVB, or none of these, along with antibiotic intervention, reduced the tumour growth significantly ($p = 0.0327$, $p \leq 0.0001$, $p \leq 0.0001$ respectively). Upon modulating the skin microbiome by antibiotic treatment, we saw an increase in commensal Clostridium species, e.g., *Lachnospiraceae sp*. ($p = 0.0008$), *Ruminococcaceae sp.* ($p = 0.0001$)., Blautia sp. (p = 0.007) and a significant reduction in facultative pathogens Corynebacterium sp. ($p = 0.0009$), *Pelomonas sp.* ($p = 0.0306$), Streptococcus sp. ($p \geq 0.0001$), *Pseudomonas sp.* ($p = 0.0358$), and *Cutibacterium sp.* ($p = 0.0237$). Intriguingly, we observed a significant decrease in *Staphylococcus aureus* frequency ($p = 0.0001$) but an increase in the overall detection frequency of the *Staphylococcus* genus, indicating that antibiotic treatment helped regain the microbial balance and increased the number of non-pathogenic *Staphylococcus* populations. Our findings suggest a synergistic strategy combining microbial modulation with phototherapy could support the management of CTCL, providing a dual front in the battle against this malignancy by both enhancing therapeutic outcomes and mitigating resistance pathways.

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Biogenesis and regulation of light-harvesting systems in diatoms

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Photosynthetic eukaryotes from marine and terrestrial environments demonstrate a schism of light-harvesting pigment composition. Land plants employ chlorophyll (Chl) *a* and Chl *b* for light energy capture. In oceans, instead, the most abundant eukaryotic algal groups (diatoms, haptophytes, and dinoflagellates) use Chl *c* to replace Chl *b*. Furthermore, many of the algae contain a large amount of fucoxanthin in their light-harvesting antennae. The biosynthetic paths of Chl *c* and fucoxanthin had long remained elusive.

We recently employed genetics to reveal biosynthetic enzymes of Chl *c* and fucoxanthin (collaboratively) in the diatom *Phaeodactylum tricornutum* (Bai *et al*. 2022 PNAS; Cao *et al*. 2023 Plant Cell; Jiang *et al*. 2023 Science). Intriguingly, whereas the enzymes are conserved among unicellular algae, the multicellular brown algae (kelps) accumulate Chl *c* and fucoxanthin but lack the homologs of the diatom enzymes found. Identification of the counterpart enzymes for the biosynthesis of Chl *c* and fucoxanthin in brown algae is in progress.

In addition to light harvesting, diatoms and green plants diverge in light signal detection. Our recent findings in *P. tricornutum* reveal a photoreceptor with a LOV domain that detects high light and a bZIP domain that directly activates the

transcription of photoprotective subunits of the light-harvesting complex (unpublished); contrastingly, green plants typically have LOV domains paired with non-transcription factor domains. This research will be presented alongside our studies on pigment biosynthesis pathways.

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Phytochromes mediate depth sensing and photoacclimation in marine diatoms

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Light is one of the most informative environmental signals. Its perception by photoreceptor proteins enable organisms to adjust their physiology to the surroundings. Light sensing in marine environments remains largely unexplored. Marine microalgae, such as diatoms, which are a prominent group of phytoplankton, possess numerous photoreceptors sensitive to blue light, in line with the deepest penetration of this band in the water column, while violet and red ones are rapidly absorbed. They also have phytochrome photoreceptors, known in land plants, bacteria and fungi to primarily absorb red and far-red light and photoconvert between an active and an inactive form based on the ratio of these two bands. In some prasinophyte and glaucophyte microalgae, phytochromes exhibit a blue-shifted absorption spectrum, interpretated as an adaptation to the predominant wavelengths in the ocean.

By combining different approaches, we investigated the light sensing properties and the possible roles phytochromes play in diatoms. We found that diatom phytochromes (DPH) of different taxonomic and geographical origins, exhibit a conserved red/far-red absorption spectra. In the molecular model species *Phaeodactylum tricornutum,* we established a DPH-response reporter system and found that DPH triggers photoreversible responses not only depending on the red/far-red wavebands but across the entire visible light spectrum, with a strong influence of blue, green, and red bands. Quantification and modeling of these responses in oceanic light fields predicted a DPH-dependent regulation with depth. Functional investigations of diatom photophysiology in cells acclimated to « depth »- or « surface »-like conditions revealed that DPH can regulate photosynthesis acclimation at depth. Combined with analysis of the biogeography distribution pattern of *DPH*-containing diatoms, mostly located in temperate and polar regions, we hypothesized that DPH could have an adaptive function in coping with light variations associated to vertical displacements in waters characterized by strong seasonal variations of the mixed layer depth.

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Study of the biodiversity in photosynthetic light harvesting and regulation in cyanobacteria

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Phycobilisomes (PBS) are the intricate light-harvesting antennas found 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. Recently, the PBS and the complex OCP-PBS structure have been elucidated from the model organism *Synechocystis* PCC 6803 at overall resolutions 1.6- 3.5 Å. The structures revealed the existence of three different conformational states of the antenna, including two previously unknown for the unquenched PBS. The PBS-OCP complex showed four OCPs organized as two dimers that quench the PBS. In our current work, we are biochemically characterizing OCP from three different marine *Synechococcus* strains from various ecological environments in the ocean. Additionally, we are analyzing gene expression under different conditions, such as high light or darkness, and identifying and quantifying pigment content, with a special focus on carotenoids, under these conditions. To date, OCP proteins have been well studied in freshwater cyanobacterial models; however, little is known about marine OCPs. Our bioinformatics analysis revealed that OCP from marine *Synechococcus* is relatively poorly conserved, with only 65% sequence identity to the OCP1 of freshwater strains (typically 85-90% identical), making them the most divergent OCP. We hypothesized that differences in primary structure would be reflected in photoconversion kinetics.

Recently, new homologous families of the constituent domains of OCP have been identified (Melnicki et al. 2016). Nine different clades of N-terminal domain homologs have been described across diverse cyanobacteria species and are named Helical Carotenoid Proteins (HCPs). Homologs to the C-terminal domain (CCPs) have also been found in nearly every genome encoding an HCP. Most likely, OCP was derived from a combination of HCP with CCP forming a single polypeptide. We are characterizing the OCP-related system of another marine cyanobacteria, *Cyanothece* ATCC 51142.

Altogether, our on-going research will shed light on the regulation of photosynthesis and photoprotection in these ecologically relevant species.

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Phototaxis and motility in natural and synthetic communities

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Phototrophic biofilm communities in most environments experience major changes in light levels throughout a diel cycle. We examined photo motility in two related cyanobacterial isolates (*Synechococcus sp.)* from thermal springs in Yellowstone National Park. Both isolates exhibited phototaxis and photokinesis but with differences in speed and motility bias and responses to specific wavelengths. The repertoire of photoreceptors and signal transduction elements in both isolates were examined.In conjunction with *in situ* observations, we suggest that phototactic strategies may be versatile and tuned to the light and local environment. In this context, we have attempted to model the collective behavior of cyanobacteria in unicellular cyanobacteria to predict phototaxis under different conditions. We also developed a binary consortium using *Synechococcus* OS-B' (Syn OS-B) and the filamentous anoxygenic phototroph Chloroflexus MS-CIW-1 (Chfl MS-1).Chfl MS-1 formed bundles of filaments that moved in all directions with no directional bias to light while Syn OS- B' exhibited positive phototaxis. This binary consortium displayed cooperative behavior: moving further than either species alone and formed ordered arrays where both species aligned with the light source. The binary consortium produced more adherent biofilm than individual species, consistent with the close interspecies association revealed by electron microscopy. Using new techniques of microscopy, microfluidics, mutants, metabolomics and transcriptomics coupled with our current analyses may give us a more predictive understanding of spatial organization and how phototrophic communities build stratified biofilms

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Photochemistry of DNA: The role of lesions

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The natural DNA bases are highly resistant to UV excitation as they dissipate more than 90% of their energy through efficient nonradiative channels leading to the ground state. Nonetheless, it is now well established in the literature that small structural changes might drastically modify the photochemical properties of DNA by lengthening the excited states lifetime and/or increasing intersystem crossing efficiency.

During this last decade, our group has focused its attention on studying DNA lesion photobehavior. The photochemistry of DNA damages is indeed of utmost importance as some of them are able to absorb in the UVA-UVB region and behave as a potential intrinsic photosensitizer. Here, we will discuss the photophysical and photochemical properties of damages such as the (6-4) photoproducts,[1]-[2] 5-formylpyrimidine derivatives,[3]-[5] or etheno adducts[6],[7] to evaluate if they fulfil the basic requirements of a good DNA photosensitizer: (*i*) to absorb in the UVA-UVB region, (*ii*) to populate efficiently their triplet excited state and (*iii*) to be able to interact with DNA components through a Type I or II process and/or a triplet-triplet energy transfer.

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Reversible photoregulation of G-quadruplex DNA structures by non-covalent azobenzene derivatives

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G-quadruplexes (G4) are non-canonical DNA structures involved in important cell regulatory functions associated with their folding mechanism. The design of small ligands capable of modulating their formation/stabilization is therefore of growing interest for the development of new anti-cancer therapies. In particular, the reversible control of G4s using bistable photoswitches offers promising perspectives for applications in photopharmacology and DNA nanotechnology, but remains largely unexploited [1]. It

has long been demonstrated that the folding/unfolding of human telomeric (HT) G4 sequences can be induced by azobenzene-derived photoswitches [2]. However, the dynamics and mechanisms underlying these processes have never been investigated. Here we present a comprehensive study of complexes made of non-covalent azobenzenes bearing quaternary ammonium substituents (AZO) with different G4 sequences, by using a combination of stationary and time-resolved optical and chiroptical spectroscopic methods. This study revealed a non-cooperative binding mode of AZO with HT G4 sequences of the type 5'-GGG(TTAGGG)$_3$-3' and the thrombin-binding aptamer G4 sequence, 5'-GGTTGGTGTGGTTGG-3' (TBA), in the absence of physiological cations. The binding of AZO to DNA induces the formation of parallel G4 topologies that can be reversibly unfolded under UV/visible excitation without noticeable fatigue. Femtosecond transient absorption measurements show that the isomerization of AZO is slowed by a factor of 4 in the presence of G4 (62ps vs. 16ps), while millisecond time-resolved circular dichroism provides evidence that G4 unfolding takes place within a few tens of milliseconds [3].

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Photobehavior of gefitinib and its photoactive metabolites in solution and in protein media

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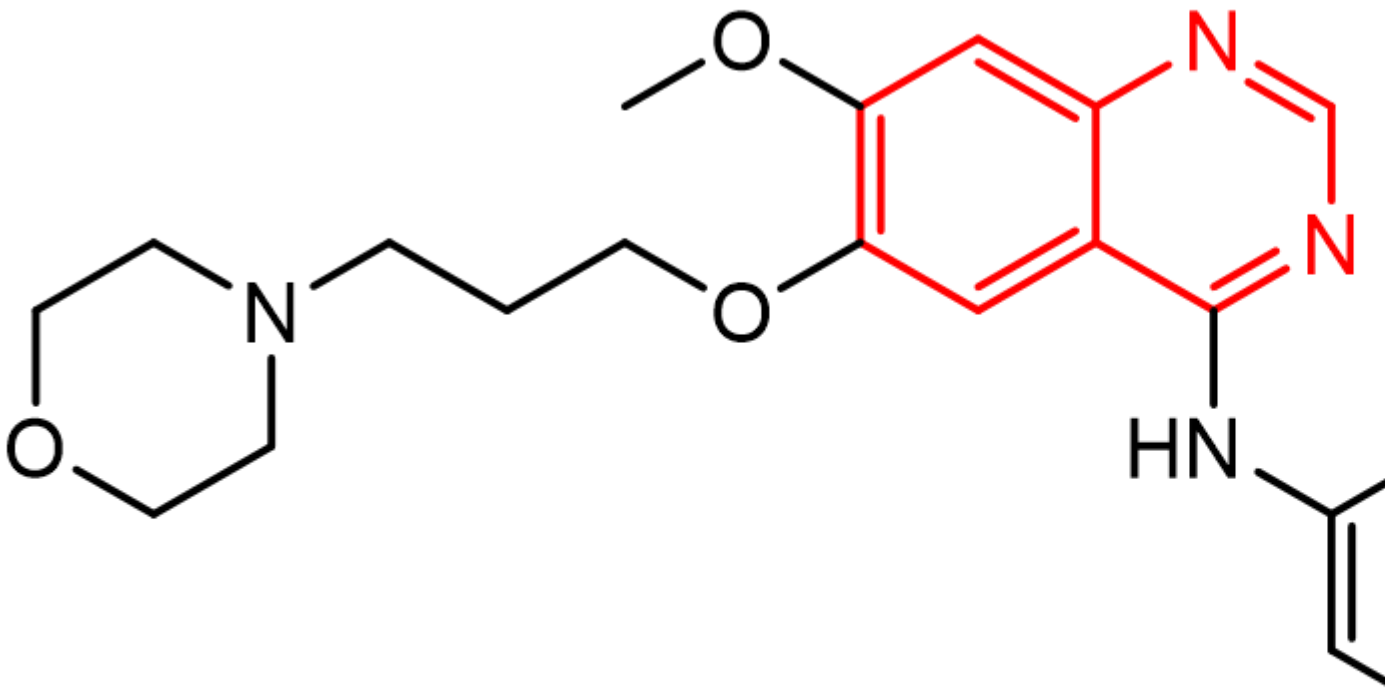
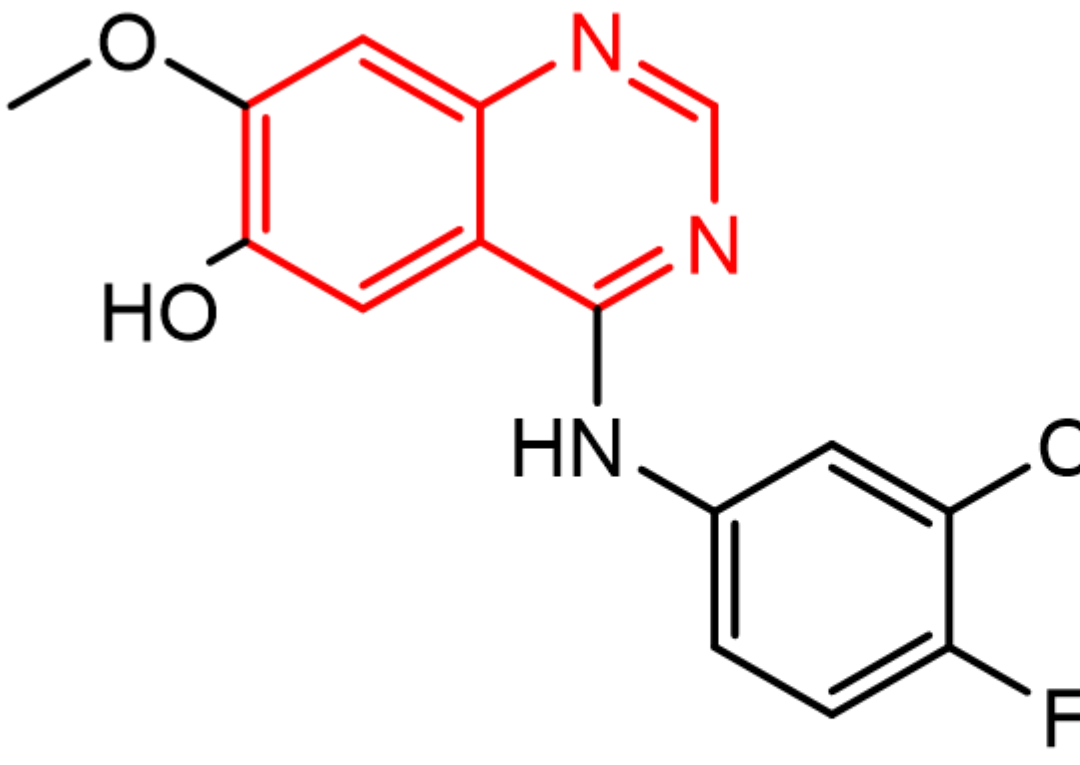
Gefitinib (GFT) is a tyrosine kinase inhibitor used to treat advanced and metastatic non-small cell lung cancer. It is metabolized *via* CYP3A4 to form a variety of derivatives including the phenolic metabolites *O*-desmorpholinopropyl gefitinib (GFT-M1) and *O*-desmethyl gefitinib (GFT-M2). Both the drug and its metabolites have recently revealed to be phototoxic; the associated mechanism is related with the excited species that are formed upon irradiation of the supramolecular drug or metabolite@protein complexes with UVA light.[1, 2]

The photobehavior of GFT, GFT-M1 and GFT-M2 has been investigated in solution and in the presence of transport proteins of human plasma, *i. e.* serum albumin (HSA) and α_1-acid glycoprotein (HAG). To this end, fluorescence spectroscopy, both in the steady-state and time-resolved modes, in addition to transient absorption spectroscopy have been used.

In general, excitation of the drug or its metabolites led to formation of locally excited (LE) states in non-polar organic solvents, whereas intramolecular charge transfer (ICT) states are formed in polar ones. By contrast, a different behavior has been observed in the confined environment provided by the protein. For GFT in complex with HSA or HAG, LE singlet states are mainly formed. However, since GFT-M1 is a phenol, excited state proton transfer (ESPT) to form phenolate-like excited species might become an alternative deactivation pathway in HSA. Conversely, locally excited (LE) states were also formed within HAG. The reserve was true for GFT-M2, which despite being also a phenol, led mainly to formation of LE states within HSA, and phenolate-like species (with a minor contribution of LE) inside HAG.[1-3]



GFT



The experimental findings are satisfactorily explained by molecular dynamics (MD) simulations. In general, the differences observed in the photobehavior of the drug and its two photoactive metabolites in protein media are consistent with their relative photosensitizing potentials.

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Shedding Light on Photoreactivity of Photosensitizer-Loaded Copolymer Micelles with Lipid Membrane Models

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The hydrophobic nature of the photosensitizer pheophorbide-a poses a challenge for its effective delivery in photodynamic therapy applications. Encapsulation within nanocarriers, such as block copolymer-based micelles, is essential to address this limitation. However, understanding the interaction dynamics between these nanovectors and biological membranes is crucial. Our study aimed to explore the mechanisms governing the interaction between copolymer micelles and membranes. We conducted physico-chemical investigations on biomimetic membranes and performed biological experiments on cell cultures. Our investigation centered on block copolymer-based micelles, particularly poly(ethyleneoxide)-*block*-poly(e-caprolactone) PEO-PCL, poly(ethyleneoxide)-*block*-poly(lactide) PEO-PLA, and poly(ethyleneoxide)-*block*-poly(styrene) PEO-PS, with liposomes.

Using the fluorescence properties of pheophorbide-a, we determined its affinity constants with both micelles and lipid vesicles, facilitating the assessment of its transfer from micelles to vesicles. We evaluated the relative production of singlet oxygen during the irradiation of pheophorbide-a, based on the type of micelles. Additionally, we monitored the leakage of a fluorescent probe from the liposomes to evaluate membrane permeability and the impact of singlet oxygen on membrane integrity. Lipid oxidation was tracked using mass spectrometry. Interestingly, although no significant differences were observed in the abilities of PEO-PCL and PEO-PS micelles to deliver pheophorbide-a to model membranes, higher concentrations of pheophorbide-a were detected in cells treated with PEO-PCL micelles. This underscored subtle differences in the delivery of pheophorbide-a through cell membranes by PEO-PCL and PEO-PS micelles.

An intriguing finding was the profound morphological transitions observed in giant unilamellar lipid vesicles upon irradiation with pheophorbide-a. This endocytosis-like process was observed exclusively when the photoactive species were encapsulated in a copolymer nanocarrier and was highly dependent on the chemical nature of the copolymer.

In summary, our study offers novel insights into the complex mechanisms governing the interaction between block copolymer-based nanocarriers and biological membranes, providing valuable perspectives on photochemical internalization mediated by nanoassemblies.

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BINDING/UNBINDING PROPERTIES OF INDOLE-BASED DIOXETANES IN DNA

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Exposure of melanin to oxidative conditions has been found to generate dioxetanes that have the potential to induce DNA damage through chemical excitation and triplet sensitization. In particular, peroxynitrite is produced in such conditions, which interacts with melanin giving rise to decomposition products such as 5,6-dihydroxyindole-2-carboxylic acid (DHICA). Subsequent reactivity between DHICA and peroxynitrite within the cell nucleus produces dioxetane derivatives, which then decompose, generating triplet excited states. These triplet states transfer energy to nucleobases, ultimately leading to pyrimidine dimerization in the DNA[1]. Other biomolecules with a similar molecular structure as DHICA and similar oxidation properties are expected to produce also dioxetanes and this type of damage under oxidative conditions. This is the case of serotonin, melatonin or tryptophan, with and indole skeleton.

Understanding the binding and unbinding interaction of the mentioned biomolecules and their dioxetanes with DNA is crucial in elucidating their relevance in the context of DNA damage. In this study, advanced sampling techniques employing the GAMBES[2] and OPES flooding[3,4] methods were utilized to compute the residence times in DNA and to characterize the binding/unbinding properties. The results obtained reveal that the release occurs on time scales of the order of μs, and the simulations have elucidated various unbinding mechanisms.

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Activation of the Mn_4CaO_5 cofactor of Photosystem II as studied by High Field EPR and MCD spectroscopy

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The structure of the S_3 state of the Mn_4CaO_5 of Photosystem II (PSII) was recently reported using high field EPR spectroscopy [1] and XFEL crystallography [2-3]. It is this 'final' meta-stable S_3 state that proceeds to O_2 formation step following a further photo-oxidation event. These data are consistent with an all octahedral Mn^{IV} complex, requiring an additional water molecule to bind to cofactor to during the S_2 to S_3 transition, but the precise mechanism of water molecule insertion remains unclear. Historically, two approaches have been used to investigate intermediates of the S-state cycle that cannot be readily trapped and characterized: i) chemical modification of the cofactor; and ii) low temperature photochemistry. Here we describe new high field EPR and MCD data targeting intermediates of the S_2 to S_3 transition.

1. High Field EPR data of chemical modified forms of the S_3 state are consistent with the cofactor adopting two, structural distinct forms. These data include Ca^{2+}/Sr^{2+} ion exchange, the binding of substrate analogs and the pH dependence of the S_2 to S_3 transition [4].
2. MCD identifies the chromophore(s) responsible for the low temperature photochemistry of the cofactor - a series of sharp bands assigned to Mn^{IV} ($^4A_2 \rightarrow {}^2E$) spin-flip transitions [5]. It is shown that these data are fully consistent with spin coupling models developed from earlier EPR/ENDOR studies and with the redox isomerism model, which explains the two S_2 state forms of the cofactor.

Together, these data suggest that the S_2 to S_3 state transition proceeds in a step-wise fashion, with cofactor deprotonation and oxidation occurring before water molecule insertion [6]. Furthermore, they support substrate water insertion being coupled to spin state conversion of the cofactor [7]. The possible extension of these same methods towards the study of the O-O bond formation step is briefly discussed.

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Real-Time Structural Changes during the S_1-S_2-S_3 state transitions of the Kok cycle of Photosystem II Caught by Time-Resolved Crystallography

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Photosystem II (PSII) catalyzes water oxidation by capturing sunlight energy at the unique pair of Chlorophylls P680 within a picosecond time scale that initiates a serial reduction/oxidation reaction between two plastoquinone, Q_A, and Q_B, a radical active tyrosine Y_Z, a catalytic manganese cluster, and substrate water molecules. This reaction is the Kok cycle catalyzed by the Mn_4CaO_5 cluster, which incorporates an extra oxygen O6 in the S_3-state to form a possible di-oxygen. The structural changes of the metal cluster and its environment during the Kok cycle have been examined at the millisecond time range. Here I will present the structural dynamics of PSII from nanoseconds to milliseconds after one or two flashes, which correspond to S_1-S_2 and S_2-S_3 transitions, respectively, using pump-probe serial femtosecond crystallography.

Y_Z, a tyrosine residue connecting the P680 and the Mn_4CaO_5 cluster, together with its surrounding amino acid residues and water molecules, showed structural changes at nanosecond and microsecond time ranges, reflecting the fast electron and proton transfer following flash illumination. Notably, one water molecule emerged and was bound to the Ca^{2+} ion in the sub-microsecond time after two flash illuminations, which disappeared later with the concomitant increase of O6, suggesting this water (O6*) is the origin of O6. There are concerted movements of water molecules in the O1- and O4-channels, protein residues, and even ligands

to complete the electron transfer, proton release, and substrate water delivery. These results provide crucial insights into the molecular mechanisms of water oxidation in PSII.

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Acknowledgment: I acknowledge many co-workers who cannot be listed due to the limited space. The work is a collaboration with several groups, including Shen Laboratory at Okayama University.

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Of spins and electrons: deciphering biological water oxidation

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The oxygen-evolving complex of photosystem II catalyzes one of the most challenging reactions in biology. Information on how the electronic structure of the tetramanganese cluster of the OEC evolves and transforms during the catalytic cycle is essential for understanding the water oxidation mechanism on a microscopic level. Here, I will discuss how quantum chemical techniques connect geometric structure with electronic structure and spectroscopy, evaluate structural models proposed by crystallography, and elaborate on possible mechanistic scenarios for further experimental investigations. Analysis of the localization of electrons on individual Mn ions and consideration of the total spin states of the exchange-coupled cluster are essential for these tasks. Our results expose limitations of current X-ray free electron laser crystallographic models of the oxygen-evolving complex in photo-advanced intermediate states,[1] while quantum chemistry in combination with X-ray absorption spectroscopy highlight specific formulations of the manganese cluster with direct implications for our understanding of catalytic progression.[2] Explicit consideration of spin coupled states in connection to electron paramagnetic resonance observations, as well as utilization of state-of-the-art correlated wavefunction approaches, shed light into the finer details of metallocofactor plasticity, on the spin dynamics that control S-state advancement, and on the sequence of events that set up the cluster for O-O bond formation.[3-5]

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Elucidating the Mechanisms of Oxyl Species Formation in Photosystem II: Insights from Computational Studies

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The catalytic production of oxygen (O_2) in Photosystem II (PSII) involves the $Mn_4CaO_{5/6}$ cluster in the oxygen-evolving complex (OEC), which progresses through a series of five states (S_0-S_4). The high reactivity of the transient S_4 state is attributed to the formation of the Mn(IV)-oxyl entity. The presence of the oxyl ligand facilitates O-O bond formation via the oxyl-oxo coupling mechanism, after which oxygen evolution occurs easily.

While observations demonstrate that the S_4 state should have an open-cubane structure, there are reports showing the possibility of an S_4 state with a closed-cubane structure. Whether the S_4 state of the closed-cubane is reactive enough toward O_2 evolution has been a subject of several studies. It has been reported that the active species in the S_4 state of the closed-cubane is a Mn4(V)-oxo species, in which Mn4 adopts a five-coordinate species with a trigonal bipyramidal geometry. As a result, if such an S_4 structure is reactive, it should drive the O-O bond formation through oxo-oxo coupling rather than oxyl-oxo coupling. This hypothesis was evaluated computationally by Guo et al., who reported that for oxo-oxo coupling to take place in the closed-cubane system with a low activation barrier, a water molecule needs to coordinate to the Mn4 atom. However, in a subsequent study, Song et al. demonstrated that the coordination of an extra water molecule to the five-coordinate Mn4(V)-oxo fragment alters the electronic identity of the complex, transforming it into an Mn4(IV)-oxyl species.

We expanded this research field and addressed some key questions left unanswered in this regard using chemical quantum methods, including: What mechanism drives the transformation of the oxo ligand into the oxyl ligand? And how does the incoming ligand facilitate the formation of such reactive key species?

We found that for the Mn4(V)-oxo in the S_4 state of the closed-cubane to become activated toward O-O coupling, it needs to change its geometry from trigonal bipyramidal to square pyramidal. This structural transition considerably stabilizes the Mn4 d_{xy} orbital, enabling an electron to transfer from the oxo ligand to the d_{xy} orbital, converting the oxo ligand into an oxyl ligand. Although the formation of the oxyl ligand sets the stage for an easy O-O coupling process, the resulting S_4 complex with a square pyramidal structure for the Mn4 fragment lies much higher in energy than that with a trigonal bipyramidal structure, making the overall barrier for O-O coupling relatively energy demanding.

However, it should be noted that the Mn4 fragment with a square pyramidal structure has an empty site for coordination. In this situation, a water molecule can coordinate to this empty site, stabilizing the S_4 state with square pyramidal geometry for Mn4. This stabilization reduces the energy gap between the Mn(V)-oxo and Mn(IV)-oxyl species, significantly lowering the overall activation barrier for oxyl-oxo coupling.

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Visualization of intercellular communication upon photo-thermal therapy in 3D tumor model

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Photo-thermal therapy (PTT) is a promising therapeutic approach for specifically targeting cancer tumors with fewer side effects due to its spatial controlled nature. Noble metal nanoparticles or metal oxides generate local heat at nanoscale upon light or magnetic field, respectively. However, cellular response in tumors to such local thermal stimuli remains unclear. In this study, we visualize intercellular communication following local plasmonic PTT stimuli in a cancer tumor model, spheroids, using a FRET-based ERK activity sensor protein. By conducting rapid 3D scanning with multi-photon fluorescence microscopy, we successfully visualized the propagation of ERK activity after PTT stimuli. Stimuli applied by a single gold nanoparticle to a single cancer cell in the 3D cellular assembly induces ERK activation in the PTT-exposed cell, which then propagates to adjacent neighboring cells. The propagation was analyzed using an AI-based program, 3DeeCellTracker, developed by Dr. C. Wen. We found that the response of ERK activity to local thermal stimuli occurs much faster than spontaneous ERK activity, with the activation signal propagation over a few cellular layers within a few tens of minutes. Since the propagation of the cellular response is much slower than heat propagation, the observed signal propagation likely occurs via intercellular communication. We are currently investigating the role of intercellular communications in enhancing PTT efficiency.

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Plasmonic nanowire single live-cell endoscopy toward intracellular material delivery

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Physically targeting specific areas within a single live cell with high spatial resolution is an attractive approach for better understanding of cellular behavior. Cell endoscopy offers a promising tool for achieving site-specific access inside a single live cell. In conventional cell endoscopy, various probes, such as glass pipettes, glass fibers, nanowires, and nanotubes, are inserted into a cell, enabling optical studies, material delivery, electrochemistry, and other applications. Optical waveguide probes are increasingly popular due to their ability to easily direct and extract optical information from the cell.

Plasmon-based single live-cell nanowire endoscopy is a promising novel tool for understanding biological processes at the single-cell level. This technique enables highly sensitive sensing of Raman signals from specific areas within a single live cell with minimal invasiveness by utilizing the unique plasmonic properties of a metal nanowire as an endoscopic probe. This allows for the study of interactions such as those between DNA and fluorescence dye inside a nucleus, or the measurement of site-specific intracellular pH. Furthermore, this technique has great potential for developing intracellular material delivery systems by engineering the surface of nanowire probes. Here, we introduce novel approaches for intracellular material delivery by integrating functional organic and inorganic molecules with plasmonic nanowires.

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Precision Assembly of Nanoparticle Superstructures using DNA

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The formation of nanocrystal assemblies, or superstructures, with optical functionality requires methods for the formation of the superstructures with high precision and in high purity. This encompasses challenges within the assembly of nanocrystals, which is a key enabling step, as well as for the full elucidation of the fundamental interactions between the component nanocrystals.

Self-assembly of nanoparticle superstructures becomes more challenging for asymmetric structures. Asymmetry can be introduced into the superstructures from asymmetry in the nanoparticle shape, the incorporation of nanoparticles of different materials or asymmetry inherent in the final geometrical arrangement of the nanoparticles with respect to one another. Asymmetry in the arrangement of the nanocrystals may take the form of large geometrical variations which, whilst remaining largely symmetrical, exhibit markedly different optical properties.

Developments in DNA nanotechnology offer control of the self-assembly of nanocrystals into discrete structures. We report an approach to construct multiple, structurally different, nanoparticle assemblies from just a few complementary nanoparticle-functionalised DNA strands. The approach exploits local minima in the potential energy landscape of hybridised nanoparticle-DNA structures by employing kinetic control of the assembly. This approach leads to the potentially facile production of a number of discrete three-dimensional isomeric assemblies of nanoparticles from a given set of ss-DNA, akin to molecular structural isomers, in extremely high (structure) yield. Using a four-strand DNA template, we synthesise five different 3D gold nanoparticle (plasmonic) tetrameric isomers. The 3D organisation of the nanoparticles is controlled by the electrostatic repulsions and steric hindrance of the charged nanoparticles.

A general method for the incorporation of different materials into DNA-based assembly is also presented, allowing access to different hetero-assemblies in high purity. The method has the flexibility to assemble nanoparticles of different sizes, shapes and

materials in the one assembly. This paves the way for a wide range of nanoparticle interactions and the optical properties of various assemblies (containing both metal nanoparticles and/or semiconductor quantum dots) will be outlined. The results show that the geometrical requirements to achieve a specifically designed coupled optical signature from a nanocrystal assembly are strict. Additionally, the effect of the interparticle separation, able to be modulated within these assemblies, will be discussed. These results have implications for the future design and realisation of functional nanocrystal superstructures.

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Light-enhanced $VEGF_{121}$/rGel induce immunogenic cell death and increase the antitumor activity of αCTLA4 treatment

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Background: Photochemical internalization (PCI) is a drug delivery system, based on PDT. The PCI technology is developed to rupture endocytic vesicles and release the drugs accumulated in these vesicles into the cytosol. Gelonin is a highly toxin protein toxin that has documented to accumulate in endocytic vesicles and activated by PCI. PCI is long known to generate an immune response in murine models and was recently shown to enhance the cellular immune response of a vaccine in a clinical study. In the present work we evaluated PCI in combination with the vascular targeting toxin $VEGF_{121}$/rGel ($VEGF_{121}$ linked to recombinant gelonin) with respect to induction of immune-mediated cell death as well as *in vitro* ICI enhancement.

Methods: DAMP signaling post $VEGF_{121}$/rGel-PCI was assessed in CT26 and MC38 murine colon cancer cell lines. Hypericin-PDT, previously indicated as an highly efficient DAMP inducer (but difficult to utilize clinically), was used as a control. ATP release was detected by a bioluminescent kit while HMGB1 and HSP90 relocalization and secretion was detected by fluorescence microscopy and western blotting. $VEGF_{121}$/rGel-PCI was further investigated as an αCTLA enhancer in CT26 and MC38 tumors by measurement of tumor growth delay. CD8+ Dependent efficacy was evaluated using a CD8+ antibody.

Results: $VEGF_{121}$/rGel-PCI was shown to induce increased DAMP signaling as compared to PDT and $VEGF_{121}$/rGel alone and the magnitude was found similar to that induced by Hypericin-PDT. Furthermore, a significant CD8+ dependent enhanced αCTLA-4 treatment effect was observed when $VEGF_{121}$/rGel-PCI was used as an adjuvant in both tumor models.

Conclusions: $VEGF_{121}$/rGel-PCI describes a novel concept for ICI enhancement which induces a rapid CD8+ dependent tumor eradication in both CT26 and MC38 tumors. The concept is based on the combination of intracellular ROS generation and vascular targeting using a plant derived toxin and will be developed towards clinical utilization.

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Targeting and blocking the PD-L1 immune checkpoint in pancreatic cancer using self-penetrating, light-responsive liposomes.

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Immunotherapy in pancreatic cancer suffers tremendously from an immunosuppressive microenvironment that is concurrent and, in part, due to extensive desmoplasia. In this work, we present light-responsive liposomes targeted towards the PD-L1 immune checkpoint in pancreatic cancer. Upon light activation, these liposomes induce immunogenic cell death and significantly disrupt tumor collagen to enable self-delivery thought pancreatic tumors developed from genetically engineered mouse model cell isolates. We will discuss the implications of self-delivery of these PD-L1 targeting and blocking liposomes and how the extent of self-delivery through tumors directly correlates with responses to immune checkpoint therapy and overall survival in pancreatic cancer.

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The Crucial Role of Atropisomerism in Enhancing Amphiphilicity and Cellular Internalization of Photosensitizers

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The ability of photosensitizers to interact with and penetrate cell membranes is crucial for treatment effectiveness, particularly in cellular Photodynamic Therapy (PDT) protocols involving longer intervals between photosensitizer administration and target tissue illumination. These protocols facilitate photosensitizer accumulation in tumors, providing sufficient time for extravasation and cellular uptake.

Redaporfin, a halogenated sulfonamide bacteriochlorin photosensitizer, is characterized by its intense absorption in the NIR region (~750 nm), high phototoxicity, and immunostimulatory properties. However, due to its macromolecular nature (MW: 1134.11 g/mol), redaporfin faces challenges related to cellular internalization (with *in vitro* studies requiring an incubation a 20-h incubation) and diffusion across the tumor mass, especially in tumors with a dense extracellular matrix and/or restricted vascularization [1].

Redaporfin exists as a mixture of four atropisomers, which differ in the spatial orientation of their sulfonamide groups relative to the macrocycle. Our research has revealed that these atropisomers exhibit markedly different cellular uptake profiles, both *in vitro* and *in vivo*, significantly affecting their PDT efficacy. The α4 atropisomer, with all sulfonamide groups on the same side of the macrocycle plane, shows the highest cell uptake and consequently the most potent PDT effects. This enhanced uptake is likely due to its higher amphipathic nature, which facilitates its interaction with the polar head groups of phospholipids on the cell membrane surface, followed by its flipping into the inner layer of the cell membrane, and passive diffusion into the cell [2].

These findings demonstrate that properly oriented polar groups can be strategically incorporated into drug design as effective cell-penetrating motifs. This was further validated by synthesizing novel porphyrin photosensitizers with strapped moieties to restrict the rotation of polar groups and force different atropisomer configurations. Notably, the cis-αα porphyrin, resembling the α4 redaporfin configuration, exhibited enhanced cellular internalization compared to the other conformers [3].

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Cell-penetrating peptide/photosensitizer conjugates for photo-triggered cytosolic delivery of RNAs and peptides

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Here we present examples of photoresponsive molecules that photo-dependently enter the cytoplasm based on the principle of photochemical internalization, and how they have been developed and used. The photoresponsive molecules discussed here are complex molecules consisting of a photosensitizer, a cell-penetrating peptide, and a cargo molecule (molecule to be delivered into the cytoplasm). When administered to cells, this photoresponsive molecule enters the cell by endocytosis and becomes trapped within endosomes, but when exposed to light, it escapes the endosomes and functions in the cytoplasm. Examples of these molecules include photoresponsive RNA carriers [1, 2] and photoresponsive apoptosis-inducing molecules [3]. Among them, we report a method (Photoinduced Cytosolic Dispersion of RNA (PCDR) method) for cytosolic delivery of small RNA molecules such as short hairpin RNA (shRNA) and microRNA (miRNA) using the photoresponsive RNA carrier. Specifically, we present the mechanism of PCDR [4, 5], RNA transfection triggered by light of different wavelengths [6], and spatio-temporally photo-triggered RNAi [7]. In addition, we report on the analysis of cellular functions by photoresponsive complex molecules [8] and discuss their potential for medical applications [9].

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Photochemical immune stimulation of melanoma

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Pigmented melanoma has not been considered amenable to PDT because of the high attenuation of the treatment light by melanin. We have developed a novel treatment protocol, using optical clearing and a dual-photosensitizer (tumor cell and vascular targeting) that was successful in eradicating thin (<1mm) pigmented melanoma grown intradermally in immunodeficient mice. In

immunocompetent mice, the same treatment resulted in time- and organ-dependent immune biomarker upregulation, eradicated tumors >4mm thick (far beyond the reach of the light to destroy tumor directly) and markedly increased survival. Further, subsequent re-challenge by *i.v.* injection of melanoma cells at 1 month post treatment resulted in no tumor formation, the tumor-specific immunity was transferrable to other mice and the response of bilateral tumor showed an abscopal effect. Direct imaging in a dorsal skin window chamber model showed immune cell infiltration into the tumor mass. Preliminary work has shown that anti-tumor response can be induced even in intraocular uveal melanoma, despite the immune-privileged status of the eye.

This phenomenon of "PhotoChemical Immune Stimulation" was then tested in other solid tumor models and using porphyrin-lipid nanoparticles (Porphysomes) that have shown high PDT efficacy in multiple preclinical tumor models. Again, marked upregulation of immune biomarkers was observed, even in tumors that are considered immunologically "cold".

This new paradigm of oncologic PDT has potential both to improve the response of primary solid tumors and to address the major challenges of tumor progression and metastases.

Brian C Wilson, Princess Margaret Cancer Centre-University Health Network/University of Toronto, CA

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Balancing Photon Harvesting Between Photosystems

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Photosystem I (PSI) and Photosystem II (PSII) are responsible for the absorption of light energy and its conversion into chemical energy, yet they absorb different wavelengths of light. Moreover, the light spectrum is not the same everywhere and it also changes throughout the day. Since PSII and PSI work in series, maintaining an excitation balance between them is essential for optimal photosynthetic efficiency and avoiding photodamage. How do photosynthetic organisms achieve this?

In this presentation, I will discuss the mechanisms by which the photosynthetic apparatus of algae, plants, and cyanobacteria adapts to changes in light color.

Understanding these adaptive responses provides insights into the resilience and efficiency of photosynthesis under variable light conditions, which has implications for improving crop productivity and understanding plant responses to climate change.

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Biodiversity of Nonphotochemical Quenching

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Non-photochemical quenching (NPQ, also called qE) is widely present in photosynthetic species. It dissipates excess light energy under adverse growing conditions, thus protecting phototrophs from photodamage. The NPQ systems employed by cyanobacteria, algae, and higher plants are all very different, whereas within a specific phylum, it seems rather conservative. Three types of NPQ systems have been identified so far: they are the blue-green light-sensitive phycobilisome-orange carotenoid protein (OCP) system in cyanobacteria, the low luminal pH-triggered LHCSR/LHCX system in green algae and diatom, and the PsbS-VDE system for higher plants. Despite decades of studies of NPQ from multi-disciplinary aspects, their quenching mechanisms remain controversial. Besides, if there is any novel type of NPQ system, it still needs to be explored. Deciphering the mechanisms of these three identified NPQ systems or searching for new NPQ gene elements or systems is essential for optimizing photosynthesis and further improving crop environmental fitness.

In the first part of this talk, I will review the up-to-date knowns and unknowns about the key players of the NPQ process and how they function together under the frame of NPQ biodiversity, covering an extensive range of photosynthetic species of cyanobacteria, green algae, red algae, glaucophyte, diatom, liverwort, and higher plants. In the second part, I will mainly share two of our recent results in our lab: one is on NPQ in the green alga of *Chlamydomonas reinhardtii*, in which we have managed to resolve the quencher molecule and unravel the quenching kinetics. The other one is on NPQ in the liverwort of *Marchantia polymorpha*. We have constructed the knock-out mutants of PsbS and VDE, respectively, and their double mutants. We found that the NPQ traits in liverwort almost mirror the ones in higher plants, showing that the PsbS-VDE NPQ systems had already fully evolved in non-vascular plants.

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Biophysical Insights into Light Harvesting Acclimation and Regulation in Oxygenic Photosynthesis

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Light harvesting complexes are crucial for supplying the reaction centers of photosystems I and II (PS and PSII) with sufficient excitation energy to sustain photosynthesis. Available excitation energy depends, of course, on the intensity and spectral composition of the light but also on the composition and organization of the light-harvesting antenna systems. The light-harvesting capacity of a photosystem can be approximated by the product of two parameters: its absorption cross section, which becomes larger as the antenna system size increases, and the quantum efficiency of charge separation, which usually decreases as the antenna size increases.

For optimal performance in specific light conditions, photosynthetic organisms often adapt the antenna systems of PSI and/or PSII on a short (regulatory) and long (acclimatory) time scale by changing either or both of these parameters. Examples of such dynamic processes include state transitions (ST), which balance the excitation energy between PSI and PSII, and nonphotochemical quenching (NPQ), a protective mechanism that dissipates excess energy to prevent photodamage.

Remarkably, the underlying mechanisms of these processes can vary significantly among species, reflecting the rich biodiversity of photosynthetic organisms. With the help of advanced spectroscopy and microscopy techniques, these processes can now be studied *in vivo* over a broad time scale, i.e. from (sub)picoseconds to days.

In this presentation I will show several recent results from our laboratory, some of which challenge long-standing views on different regulation mechanisms. These insights, drawn from a variety of organisms, underscore the complexity and diversity of strategies employed in light energy management in oxygenic photosynthesis.

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Diversity and evolution of far-red light photoacclimation in cyanobacteria

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The discovery of cyanobacteria capable of harvesting far-red light has changed the paradigm that oxygenic photosynthesis is only driven by visible light and exclusively by chlorophyll *a*. There are two known types of far-red photosynthesis. Firstly, a constitutive adaptation that uses a majority of chlorophyll *d,* which is restricted to a single genus (*Acaryochloris*). Moreover, an acclimation response, known as Far-Red Light Photoacclimation (FaRLiP), which uses chlorophyll *f* and is present in phylogenetically diverse cyanobacteria (1). FaRLiP involves the extensive remodelling of the photosynthetic machinery, via a cluster of approximately 19 *genes coding for* paralogous subunits of Photosystem I, Photosystem II, phycobilisomes and master control elements. Here, I will highlight the similarities and differences of FaRLiP among cyanobacteria on a cell, membrane, protein and DNA level by using bioinformatics, biochemical and biophysical methods. Our study focuses on cyanobacteria of the genus "*Chroococcidiopsis*" (2), as well as the phylogenetically early-branching group of "*Halomicronema*/*Nodosilineales*". The latter group is especially underrepresented. We could increase the number of FaRLiP cyanobacteria among them by using stringent far-red cultivation methods on samples from the hypersaline environment of the Sebkha Ooum Dba (Morocco). This data enabled high-resolution phylogenetic work and supports that FaRLiP appeared early in cyanobacterial evolution (3). Furthermore, strains were discovered that only contain a partial FaRLiP clusters, without genes for a far-red PSI variant, but with a normal growth behaviour under far-red light (4) and without genes for a far-red PSII. These "outliers" have been characterised by biophysical and biochemical methods, raising the question of the minimal requirements for FaRLiP.

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Using blue light to control transpiration: improving growth in low VPD

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Aims: In protected cultivation, a microclimate with low vapour pressure deficit (VPD) is common due to limited ventilation, high plant density and low air movement. The aim of this study was to investigate how blue light (BL) interacts with VPD on growth, stomatal regulation and transpiration rate in light and darkness. To improve the understanding of signals involved, changes in abscisic acid (ABA) metabolism and content of carbohydrates and flavonoids were studied.

Methods: Cucumber plants (*Cucumis sativus* 'Quatro') were exposed to moderate (1.12 kPa) and low VPD (0.28 kPa) in combination with a photosynthetically active radiation of 200 µmol m -2 s -1 with either 5% or 30% BL.

Results: Additional BL reduced the ABA content (+ its metabolites) during light and darkness, increased the adaxial stomatal density, transpiration rate and foliar nitrogen (N) content irrespective of VPD. ABA regulation was not affected by VPD, but the amount of BL provided during the day affected the ABA catabolic pathway at night. In the high BL (30%) treatment, higher transpiration and higher content of ABA-glucose ester (ABA-GE) - a storage form of ABA - were found during night. By contrast, in low BL (5%), more ABA was converted to phaseic acid (PA). Increased starch degradation in response to increased BL resulted in a higher content of transport carbohydrates such as sucrose, raffinose and stachyose in source leaves and was accompanied by a higher fruit appearance rate in low VPD, in contrast to moderate VPD where additional BL reduced the fruit appearance rate.

Conclusion: Our results provide new insight into the regulation of ABA under different VPDs and BL proportions and show that additional BL act as a weak stressor in moderate VPD but can be a cultivation strategy in low VPD to increase growth through increase in transpiration and N content.

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Assessing temperature and light interactions on non-photochemical quenching in microalgae

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Understanding the effects of environmental stressors on photosynthetic organisms is crucial for predicting their responses to climate change. In this work we utilized advanced phenotyping techniques to investigate how temperature and light gradients affect non-photochemical quenching (NPQ) in microalgae.

The research introduces the "Phenoplate" method, which simultaneously assesses the impact of temperature and light gradients on marine microalgae's NPQ responses under chemical stress. Rapid light curves were employed to measure the photoprotective mechanisms of *Tetraselmis sp.*, T*halassiosira pseudonana*, and *Nannochloropsis oceanica* across a temperature gradient and varying phosphate levels. The results revealed that photoprotective mechanisms were highly efficient at lower temperatures, while higher temperatures negatively impacted the relaxation of photoprotection in *Tetraselmis sp.* Unique NPQ signatures were observed in *Thalassiosira pseudonana* and *Nannochloropsis oceanica*, indicating species-specific responses to temperature and light interactions [Herdean et al., 2022].

Additionally, the work maps the temperature dependency of NPQ in *Chlorella vulgaris* using the same Phenoplate technique. It demonstrated that fast-relaxing NPQ (qE) follows an inverse normal distribution with respect to temperature and is insensitive to prior temperature acclimation. A slow-relaxing NPQ component displayed a normal distribution similar to quantum yield (Y(II)), peaking at higher temperatures. These findings highlight the strong temperature dependency of photosynthetic processes even in conditions where temperature changes at short time scales (minutes). Furthermore, our findings highlight that the impact of temperature in PAM measurements may have been underestimated and suggest that results from experiments performed “room temperature” may be misleading [2].

This research underscores the importance of considering multiple environmental factors simultaneously to understand the complex responses of photosynthetic organisms.

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Photo-modification of Eumelanin and Pheomelanin and Its Biological Implications

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Melanocytes produce two types of pigment, eumelanin and pheomelanin. Eumelanin consists of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA), while pheomelanin consists of benzothiazine and benzothiazole units. It is generally accepted that eumelanin is photoprotective for pigmented tissues while pheomelanin is phototoxic. In this review, we summarize current understanding of how eumelanin and pheomelanin structures are modified by ultraviolet A (UVA) and also by visible light and how reactive oxygen species participate in those processes. Alkaline hydrogen peroxide oxidation (AHPO) was employed to characterize DHICA-derived eumelanin and benzothiazole-type pheomelanin, giving pyrrole-2,3,5-tricarboxylic acid (PTCA) and thiazole-2,4,5-tricarboxylic acid (TTCA), respectively. Reductive hydrolysis with hydroiodic acid gives 4-amino-3-hydroxyphenylalanine (4-AHP) and 3-AHP from the benzothiazine moiety of pheomelanin. Analyses of natural and synthetic melanins show that the photoaging of eumelanin gives rise to Free PTCA (produced by peroxidation in situ) and pyrrole-2,3,4,5-tetracarboxylic acid (PTeCA, produced by cross-linking), leading to the increases in the ratios of Free PTCA/Total PTCA and PTeCA/PTCA. In pheomelanin, the TTCA/4-AHP ratio increases and the 4-AHP/3-AHP ratio decreases with photoaging, indicating the conversion of benzothiazine to the benzothiazole moiety. Analysis of those markers and their ratios show that both eumelanin and pheomelanin are photo-modified in human hair, alpaca fiber, cultured melanocytes, human retinal pigment epithelium melanosomes, and human ex vivo skin. Using synthetic melanins, we also found that singlet oxygen, in addition to superoxide anions, is photogenerated and quenched upon UVA irradiation. The biological implications of those findings are discussed in relation to the tanning process, to melanomagenesis in the skin and to age-related macular degeneration in the eyes.

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Modification of DNA photodamage by melanin in human skin *in vivo*

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Solar ultraviolet radiation (UVR) induces DNA damage in human epidermis especially the cyclobutane pyrimidine dimer (CPD) that may lead to skin cancer, the incidence of which is much lower in black compared with white skin. It is assumed that melanin inhibits skin cancer by preventing CPD formation, but we lack quantitative data.

We compared CPD in black and white skins after acute erythemally equivalent exposures of solar simulated radiation (SSR). CPD were assessed in three zones: basal, mid and upper epidermis [1]. A quantitative analysis showed a strong relationship between CPD and melanin concentration. The high concentration of melanin in the basal layer offered a CPD protection factor of 59 compared with 5 in the upper epidermis with the least melanin. Importantly, the basal layer is the region that contains

keratinocyte stem cells and melanocytes. The level of CPD protection by melanin in the basal layer is comparable to differences in skin cancer incidence in black and white skins and provides indirect evidence that melanin reduces skin cancer incidence by inhibiting CPD.

SSR can also induce “dark” CPD which are formed after the end of exposure [2]. There is evidence that melanin carbonyls act as sensitisers for “dark” CPD formation [3]. White skin showed a similar “dark” CPD distribution in the three epidermal zones described above. In contrast in black skin there was a high prevalence of “dark” CPD in the upper epidermis, some in the mid epidermis but none in the basal layer. This suggests that melanin may sensitise or inhibit “dark” CPD depending on its concentration.

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Photosensitization of melanin in skin cells and in hair fibers

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Although melanin is capable of efficiently absorbing and scattering electromagnetic radiation, melanin itself, as well as its precursors, are also capable of generating free radicals and other oxidizing species, such as singlet oxygen ($^1O_2$), after absorbing photons in the UV and visible ranges. In addition to generating several reactive species by electronic excitation, melanin also acts as a sacrificial antioxidant agent, by reacting and neutralizing these species [1]. ^{1}O2, for example, can add to the indole rings present in the structure of melanin, forming hydroperoxides and causing the photobleaching of melanin. The generation of $^1O_2$ in hair fibers depends on the amount and type of melanin, and can explain the color change that happens after persistent sun exposure [2], as well as, it can be used to develop novel methodologies to precisely reduce hair color, without affecting significantly the mechanical properties of the hair fiber and of the cuticle structure [3]. In human skin, melanin is one of the most important protecting agent against the excess of sun exposure. However, melanin is also involved in excited state and free-radical reactions. Skin of color (SOC) individuals synthesize eumelanin granules in greater amount and bigger size than fair-skinned people, being better protected against UVR, but being susceptible to several skin disorders, including melasma, which can be correlated to the effects of melanin photosensitization with Visible Light (VL). VL can damage melanocytes through melanin photosensitization and $^1O_2$ generation, thus decreasing cell viability, increasing membrane permeability, and causing both DNA photooxidation and necro-apoptotic cell death. UVA (355 nm) and visible (532 nm) light photosensitize $^1O_2$ with similar yields, and pheomelanin is more efficient than eumelanin at generating $^1O_2$ and in resisting photobleaching. Although melanin can protect against the cellular damage induced by UVB, exposure to VL leads to mutagenic DNA lesions (i.e., Fpgand Endo III-sensitive modifications) [4]. These data demonstrate the types of damage that an overexposure to VL can cause in human skin. Novel sun screen technology should favor broad spectrum protection and personalized formula, especially for SOC people that needs stronger photoprotection in the visible range, compared with fair-skin people that needs stronger protection in the UVB [1].

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Photooxidaton of eumelanin affects its efficiency to photogenerate and quench singlet oxygen

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Photoprotective action of melanin is commonly ascribed to its optical properties responsible for the efficient absorption of ultraviolet and visible light, ultrafast conversion of energy of the absorbed photons into heat, which minimizes the formation of reactive oxygen species, and the ability of melanin to scavenge oxidizing radicals and quench singlet oxygen. Although natural melanins were previously viewed as very stable pigments showing little metabolic turnover, it is now recognized the pigments can undergo with aging substantial physicochemical changes. Recently, we have demonstrated that in vitro photoaging of human hair melanosomes significantly modified their photochemical reactivity [1]. To address this issue comprehensively, a thorough analysis of selected physical and photochemical parameters of a synthetic DOPA-melanin, subjected to aerobic photolysis or treatment with hydrogen peroxide, was carried out [2]. Physical properties of the studied melanin were assessed by UV-vis absorption, EPR spectroscopy, and DLS. Molecular changes of the melanin undergoing photochemical or chemical bleaching were analyzed by alkaline hydrogen peroxide oxidation. Aerobic photoreactivity of the melanin was determined by EPR-oximetry, EPR-spin trapping, and time-resolved singlet oxygen phosphorescence. Changes in antioxidant properties of the melanin were monitored by DPPH EPR assay. The data show that photochemical and chemical treatment of the synthetic eumelanin caused its dose-dependent bleaching and irreversible modification of the melanin paramagnetic, electron- and ion-exchange properties. Bleached melanin exhibited enhanced efficiency in photogeneration of singlet oxygen and reduced antioxidant properties. It is

postulated that oxidative modifications of melanin, accompanying aging, could affect its photoprotective functions and stimulate phototoxic behavior.

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Molecular beacons as optical switching probes for intracellular theranostics and optical biosensing

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Molecular beacons (MBs) are suitable molecules capable of turning on or modifying their light emission only after a molecular interaction with well-defined molecular targets [1]. MBs are formed by oligonucleotide sequences and are generally labelled at the two strand ends with a fluorophore F on one side and a quencher Q on the other side, and structured in a hairpin shape capable to emit fluorescence only in the presence of a complementary oligonucleotide sequence which opens the hairpin structure.

Extremely interesting is the use of this nanosensing probe at intracellular level, suitably coupled to intracellular cargos, for the detection of target nucleic acid - such as messenger RNA (mRNA) in cells - combining the ability of sensing specific nucleic acids with the pharmacological silencing activity preventing the overexpression of proteins associated to pathologic conditions and. Their capability to act as theranostic agents has been proved with a molecular beacon specific for survivin mRNA immobilised on polymethylmethacrylate nanoparticles able to promote survivin MB uptake in human A549 cells [2].

MBs can be also used in both fluorescence-based and SERS-based optical biosensors. In the first case, the molecular beacon capable to hybridize with the mRNA for the survivin was immobilised at the distal end of a fibre nanotip and the emitted fluorescence is modulated by the presence of the target.

In the second case the MB is used to provide a signal-off mechanism in a SERS-based biosensor for the detection of miRNA-183, a miRNA biomarker that is specific for chronic obstructive pulmonary disease. The oligonucleotide biorecognition element is immobilised on a SERS substrate on one end and labelled with a Raman reporter on the other end [3].

The purpose of this talk is to provide an exhaustive overview on the design, implementation and characterization of these complex nanostructures as optical sensing probes.

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Applications of minimalistic delocalized lipophilic cations: from nanocarriers to mitochondrial markers

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Increasing attention has been paid in recent years to the development of organic molecules designed to accumulate in cellular organelles. Mitochondria are one of the targets of current investigations [1], both in the bioimaging field and in the therapeutic area. Mitochondrial probes described in the literature share some common features, such as a delocalized positive charge along an extended aromatic organic structure. The term "delocalized lipophilic cation" (DLC) is commonly used to describe this class of mitochondria-locating structures.

Our group has focused over the past few years on studying a series of DLCs with the aim of delivering them to the mitochondria of living cells to image biologically relevant biomolecules, particularly reactive nitrogen species like nitric oxide (NO). This presentation will provide an overview of these investigations [2], highlighting not only the biosensors used for bioimaging but also related photobiological applications like the development of photoactive agents for photodynamic therapy (especially antimicrobial).

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Luminescence nanoparticles as "our spies inside" for manometry or thermometry at the biomedical arena

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There are several physical parameters intrinsically related to human health. For instance, certain temperature fluctuations can be used as an early indicator of the development of diseases, such as degenerative processes of the nervous system, acute inflammation caused by infectious agents, and cardiovascular diseases.[1] Hence, thermal monitoring of tissues and organs has emerged as a valuable tool for early detection of threatening diseases.[2,3] Among the main requirements to carry out it, it must be performed remotely, without perturbing the temperature of the tissue while measuring, also avoiding physical alterations of the organ under investigation. [4]

Luminescence thermometry represents an alternative technique that overcomes the limitations affecting other methods (invasiveness; only reporting surface temperature). It is based on the use of luminescent nanothermometers (LNThs) (nanoparticles (NPs), proteins, or dyes whose luminescence is strongly temperature-dependent) as remote thermal reporters. [5,6]. At some point LNThs have been applied for remote thermal sensing in animal models [7] enabling, for instance, non-invasive monitoring of brain activity,[8] diagnosis of ischemic tissues,[19] detection of inflammation processes, and control of thermal therapies of solid tumors.[10,11] However, *in vivo* luminescence thermometry is still not yet a completely reliable technique. Not only the presence of biological tissues in the optical path significantly reduces the amount of detected luminescence;[6] more importantly, they induce spectral distortions that yield inaccurate thermal readouts.[12] A way around these conundrums may be to switch detection of the outcoming signal from the spectral domain to the time-based one.

In this work, the robustness of relying on lifetime of the luminescence is evaluated, through near infrared nanoprobes - Ag2S semiconductor nanoparticles (NPs)- as lifetime-LNThs was initially evaluated through *ex vivo* experiments and simple numerical calculations. After ascertaining the negligible impact of tissue extinction coefficient in the lifetime-based thermal readout, the actual potential of Ag2S NPs as τ-LNThs for **thermal monitoring of internal organs**, in our case the liver, was demonstrated in an *in vivo* inflammation model.

Aside from temperature, another biomedically relevant aspect is how the mechanical forces control the function of organisms and mediate the interaction between biological systems and their environments. Knowledge of these forces will increase the understanding of biological processes and can support the development of novel diagnostic and therapeutic procedures.[13,14] Current approaches to measuring forces over a broad range of values are invasive and lack versatility. A promising way to overcome these hurdles is **luminescent nanomanometry**. Quantum dots (QDs) specifically have optical properties that depend on their size because of the quantum confinement, which makes them responsive to applied forces. Here, a thorough study is conducted on the nanomanometry performance (pressure-dependent photoluminescence) of CuInS2 QDs in the red/near-infrared range. [15, 16] That feature can enable the measurement of mechanical forces in the range of physiological relevance in a remote and minimally invasive way. It is shown that tuning size and stoichiometry can simultaneously enhance the CuInS2 QDs' brightness and response to applied pressure. Hence, this line of research is providing design guidelines on how fundamental parameters assist the quest for better luminescent nanomanometers.

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Exploring the potential of fluorescent nanoprobes for the versatile detection and quantification of nitric oxide in live cells

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Nitric oxide (NO) is involved in numerous biological processes, playing an important role in the regulation of diverse physiological and pathophysiological mechanisms of the cardiovascular, nervous and immune systems; and alterations in the intracellular NO concentrations have been linked to a large number of diseased states.[1, 2] Considering the significant role that NO plays in important biological functions, the development and improvement of methods to detect and quantify intracellular NO are essential to further our understanding of the biological roles of NO.

This contribution will present different approaches to the constructions of (nano)probes sensitive to NO[3-5] and their application for the intracellular detection and quantification of NO. Their potential to be NIR-excitable will be explored to utilise the advantage of the high photostability, high biological tissue penetration and minimal photodamage associated with this long-wavelength excitation. The (nano)probes are broadly applicable and are able to detect and potentially quantify NO levels in an extensive range of cellular environments including endogenous NO in RAW264.7γ NO^- macrophages and THP-1 human leukemic cells, and endogenous and exogenous NO in endothelial cells. The (nano)probes accumulated in the acidic organelles of the tested cell lines showing negligible toxicity. The (nano)probes will be based on gold nanoparticles and on upconverting nanoparticles (UCNPs). For the UCNP-based example, a bilayer-based strategy for the surface modification of hydrophobic nanoparticles is introduced that leads to excellent colloidal stability in aqueous environments and good protection against disintegration, while permitting surface functionalization via simple carbodiimide chemistry.[5]

Based on their excellent sensitivity and stability, and outstanding versatility, the developed (nano)probes could be applied for the spatiotemporal monitoring of *in vitro* and *in vivo* NO levels.

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The genomic landscape of melanoma-prone skin

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Normal skin carries a high burden of somatic mutations, yet this does not explain where a melanoma will form. From 3D total body photography studies of high-risk individuals, we have observed a trend toward melanoma excisions clustered in regions on the back. This posed the question – are these melanoma-prone skin regions? The PhotoMelanoma Study aimed to determine the genomic architecture of the microenvironment that favours melanoma formation. We assessed photodamaged skin adjacent to an invasive melanoma excision, photodamaged skin 5cm away, and photoprotected skin from the same individual. To address this, we invited 19 study participants from our high-risk melanoma cohort (n=300+) to donate three biopsies for genomic analysis. Each biopsy was evaluated for hotspot mutations via the ultra-sensitive droplet digital PCR system; somatic mutation burden, mutation signature, and copy number aberrations via deep panel sequencing of 300+ cancer-related genes (PanelSeq), and global methylation profiling of 900K loci. In brief - as expected, PanelSeq showed UV-related mutation signatures (SBS7) with levels similar the across sun-exposed sites, whereas signature SBS2 (APOBEC activity) was enriched (67%) at the scar-adjacent site, with the number of mutations associated with SBS2 reaching significance (Wilcoxon matched-pair; p=0.015). Global DNA methylation profiling identified 2000+ loci differentially methylated between photodamaged and photoprotected sites, including *HOX* family members. These data are part of a comprehensive genomic and transcriptomic profile to characterise melanocytes, naevi, and the microenvironment to identify the molecular triggers for melanoma formation. In sum, we have uncovered enrichment of somatic events at melanoma excision sites that may provide the soil for *de novo* melanoma development.

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Dissecting the roles of BAP1 in uveal melanoma

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BackTIL the Future: Cell Therapy for Immunotherapy Resistant Metastatic Melanoma

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Tumour Infiltrating Lymphocyte (TIL) Adoptive Cell Transfer (ACT) is a robust treatment for patients with metastatic melanoma resistant to standard immune checkpoint inhibitors (ICI) such as pembrolizumab (anti-PD1). TIL ACT involves surgical excision of tumour deposits, followed by ex-vivo expansion in specialised laboratories of high numbers of immune cells (up to 100 billion) which are infused into the patient to treat their melanoma. The M14 phase III trial compared TIL ACT to ipilimumab as second line treatment after anti-PD1 and cell therapy displayed a higher objective response rate of 49% compared to 21%. Moreover around 25% of patients treated with TIL ACT had durable responses at the 5 year landmark. Given more than 66% of patients develop resistant to ICI in the metastatic melanoma there is great need for TIL ACT in Australia.

Currently TIL ACT is only available in specialised facilities in the US and Europe. However the technical and infrastructure hurdles have been overcome with a partnership with Harry Perkins Institute of Medical Research and Cell and Tissue Therapies Western Australia (CTTWA) which is a Therapeutic Goods Administration (TGA) and Foundation for Accreditation of Cellular Therapy (FACT) Good Manufacturing Practice (GMP) approved cell manufacturing facility. The PERTIL trial will open in Q1 2025 for patients with ICI resistant metastatic melanoma and will establish TIL ACT as a locally manufactured cell therapy product to Australian patients.

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Targeting Melanoma Heterogeneity to Improve both Targeted and Immune Therapy

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Despite the success of targeted and immune therapies, many patients with advanced melanoma still die due to therapy resistance. Drug resistance is a major challenge for effective melanoma therapy. Plasticity of solid cancer plays a critical role in shaping treatment response. What determines the occurrence of phenotypically distinct tumour cell domains in solid cancers is poorly understood. Utilizing *in vitro* and *in vivo* three-dimensional models, we show that in melanoma spatial organization of plasticity is dictated by the expression and activity of the lineage-survival oncogene microphthalmia-associated transcription factor (MITF). Mechanistically, we reveal that MITF controls extracellular matrix (ECM) composition and decreases ECM organization. This leads to reduction of Rho-ROCK-myosin signalling-driven mechanotransduction through poor focal adhesion maturation and reduced contractility of the actin cytoskeleton. The resulting altered tumour microarchitecture and structural relaxation decrease tumour solid stress and subsequently $p27^{Kip1}$ expression, ultimately reducing plasticity. Consequently, selective inhibition of ROCK phenocopies the effect of MITF over-expression, demonstrating the importance of cell-ECM crosstalk in this process.

In summary, our findings place tumour cell-ECM crosstalk resulting in altered tumour microarchitecture and ROCK-driven mechanotransduction as a central driver of melanoma cell plasticity. Indeed, we show that resistance to targeted therapies is often cell cycle dependent, underlining the importance of tumour cell plasticity for successful targeted therapy. Moreover, we show that structural relaxation and decreased tumour solid stress allow deeper immune cell penetration, and thus provide potential for therapeutically targeting this phenomenon for improved immune checkpoint therapy.

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Immunological photodermatoses and photoaggravated diseases

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Photodermatoses are common skin disorders. Diagnosis may be difficult because clinical manifestations are often spontaneously self remitting and phototesting is almost always necessary. Unfortunately, special equipments that are no more available on the market are needed and there is the need of a standardization of procedures. Treating photosensitivity diseases with UV-based therapies appears at first glance paradoxical, but clinical practice proves that such methods are still leading interventions in patients with difficult-to-manage UV sensitive conditions. The idiopathic photodermatoses are the primary targets of photo therapeutic management, including polymorphous light eruption (PMLE), chronic actinic dermatitis (CAD), actinic prurigo (AP), solar urticaria (SU), and hydroa vacciniforme. For patients that do not respond to usual strategies of photoprotection, phototherapy is likely the next best option by itself or in combination with other treatments. The choice of UVB, UVA1 or PUVA depends most often not on specific therapeutic considerations but rather on availability of the hardware. Recently, there have been encouraging developments in managing CAD and SU. JAK inhibitors, which have shown encouraging results in e.g. autoimmune disease and atopic dermatitis, have also been helpful in managing otherwise non-responsive CAD patients. Omalizumab targets IgE and has been used to manage patients with SU. These developments are encouraging and we look forward to additional agents coming to enlarge the range of options for these often desperate patients. Still, the main obstacle for new therapies may be the concern for side effects and for cost. Phototherapies if used correctly have a very favorable side effect profile and costs are not excessive.

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What's New in Drug, Genetic and Metabolic Photodermatoses?

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While there are about 400 drugs that have been reported to have photosensitivity potentials (1), systematic reviewed published in 2018 showed that only vemurafenib (BRAF inhibitor for metastatic melanoma), NSAIDs and antibiotics (fluoroquinolones, tetracyclines) are supported by strong evidence. More recently, oncologic drugs [BRAF inhibitors, anti-CCR4 antibody (mogamulizumab), and EGFR inhibitors] have been shown to have phototoxic side effects.

The list of hereditary photodermatoses is long; this includes xeroderma pigmentosum, Cockayne syndrome, UV-sensitive syndrome, trichothiodystrophy, Bloom syndrome, Rothmund-Thomson syndrome, Smith-Lemli-Opitz syndrome and Hartnup disease (3). Because of the rarity of these diseases, photobiologic studies on them consisted only of small number of patients.

Among metabolic photodermatoses, the new development has been on the treatment of erythropoietic protoporphyria (EPP). Afamelanotide, analogue of human alpha-melanocyte stimulating hormone, has now been approved for the treatment of EPP (4). Dersimelagon, an oral selective melanocortin 1 receptor agonist, has also been shown to be effective in EPP (5).

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Shedding Light on Photodiagnostics

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Abnormal photosensitivity may be obvious clinically and there may be no need for photodiagnostic investigations, as with typical polymorphic light eruption. However, photosensitivity is frequently not considered as a possibility, either by the patient, their Primary Care physician or their dermatologist, leading to missed or delayed diagnosis. Accurate diagnosis is essential to advise patients and physicians regarding appropriate management, and photosensitivity diseases are a diverse group of conditions, with heterogeneity in clinical presentations and photodiagnostic findings.

Investigation through narrow waveband ultraviolet (UV) and visible light testing, such as with the use of a monochromator, provides objective evidence of the action spectrum for induction of abnormal photosensitivity, and can facilitate evaluation of treatment outcomes and the natural history of the condition. Supplementary investigations with broader band iterative UV provocation testing, patch and photopatch testing, and screening for conditions such as lupus and the cutaneous porphyrias, can enable a comprehensive diagnosis to be made. The British Photodermatology Group/British Association of Dermatologists reviewed the provision of photodiagnostic services in the UK. This highlighted the diversity of photosensitivity diseases and the complexities and challenges of investigations, and attempted to initiate a consensus approach between Centres.

However, it is recognised that there is a paucity of photodiagnostic services worldwide, and raising awareness of photosensitivity conditions and the key components of photodiagnostic services, is important for accurate diagnosis and management. This is essential as the major adverse impact of photosensitivity on patients' lives is increasingly recognised, and the provision of support through patient-centred care is a priority.

New developments in photodiagnostic techniques, with advances in LEDs and refinement of existing investigations such as photopatch testing, aim to improve accessibility, ease and efficiency of photodiagnostic services, and this presentation will shed further light on the role of photodiagnostics in the management of patients with photosensitivity diseases.

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Photoprotection and management of the photosensitivity diseases

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Photosensitivity diseases encompass a variety of conditions characterized by an abnormal skin response to ultraviolet (UV) radiation. Effective photoprotection is crucial in managing these conditions to prevent exacerbations and improve the quality of life for affected individuals.

Photosensitivity diseases, including polymorphous light eruption (PMLE), actinic prurigo, chronic actinic dermatitis, solar urticaria and porphyria, result from heightened sensitivity to sunlight radiation. Other photosensitivity disorders are caused by DNA repair defects, such as xeroderma pigmentosum, or others due to the lack of melanin, such as albinism. The cornerstone of managing these diseases is minimizing light exposure. Photoprotection strategies include the use of broad-spectrum sunscreens, protective clothing, and behavioural modifications to avoid peak sunlight hours. However, it is necessary to take into account the culprit wavelengths in every disease and in every patient to prescribe the most convenient sunscreen. In general, UVA and visible light are the most frequently involved, therefore sunscreens should contain filters and other active ingredients such as antioxidants or photoimmunoprotectors to provide better protection. Infrared radiation A (IRA) could also aggravate some of these photodermatosis. Additionally, systemic photoprotective agents, such as Polypodium leucotomos extract, have shown beneficial effects, especially in polymorphic light eruption.

Patient education is also important to have proper photoprotective behaviour using clothes, hats, sunglasses and using even protective screens and wearing dosimeters.

Regarding treatment, topical corticosteroids and calcineurin inhibitors are commonly prescribed to manage acute flare-ups, while systemic agents like antimalarials and immunosuppressants are reserved for severe cases. Solar urticaria could benefit of the use of omalizumab in recalcitrant cases. Furthermore, advances in phototherapy, including narrowband UVB therapy, have shown efficacy in desensitizing the skin and reducing the frequency of flare-ups in certain photosensitivity disorders. Genetic and molecular research is paving the way for targeted therapies that address the underlying pathophysiological mechanisms of these diseases.

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Beyond DNA repair: Novel functions of Cockayne syndrome B (CSB) and Xeroderma pigmentosum A (XPA) proteins

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CSB and XPA are inherited diseases characterized by UV hypersensitivity, high skin cancer risk (XPA), and premature aging, which are thought to be caused by defective repair of nuclear DNA (nucleotide excision repair; NER). There is, however, increasing evidence that the CSB and XPA proteins have important functions beyond their role in NER.

Using CSB-deficient human fibroblasts, *Caenorabditis elegans*, and mice, we showed that CSB promotes acetylation of alpha-tubulin and thereby regulates autophagy. At the organ level, chronic exposure of $csb^{m/m}$ mice to UVA radiation caused a severe skin phenotype with loss of subcutaneous fat, inflammation, and fibrosis. These changes were associated with an accumulation of autophagic/lysosomal proteins and reduced amounts of acetylated alpha-tubulin. At the cellular level, we found that CSB directly interacts with the histondeacetylase 6 (HDAC6) and the alpha-tubulin acetyltransferase ME-17. Administration of the pan-HDAC inhibitor SAHA improved autophagic function in CSB-deficient models from all three species, and rescued the skin phenotype in $csb^{m/m}$ mice. HDAC inhibition may thus represent a therapeutic option for this incurable disease.

Given the importance of symptoms concerning the central nervous system in CSB patients, we are currently employing human induced pluripotent (iPS) cell-derived 3-D neurospheres, brain spheres and brain organoids. In these models we find CSB-deficiency to be associated with endophenotypes, that may underlie the neuropathologies of CSB patients and that some, but not all of these endophenotypes can be improved by SAHA treatment.

XPA is another NER protein with important functions outside the cell nucleus. We have observed that XPA proteins are located inside the mitochondria, and sequencing of mtDNA, RNAseq transcriptome analysis and functional assays suggest XPA to be important for the integrity and function of mitochondria.

Taken together our studies emphasize that NER proteins have important functions beyond their role in nuclear DNA repair.

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Advancements in Solar Fuel Generation: Exploring the Frontier of Artificial Photosynthesis

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Artificial photosynthesis is considered a promising method for achieving carbon-neutral targets. The hydrogen evolution reaction (HER) from the photoelectrolysis of water and the photoelectrochemical (PEC) CO2 reduction have gathered significant attention as an effective way to store intermittent solar energy in fuels and chemicals, as well as closing the chemical carbon cycle. In this approach, light absorbers, catalysts, contacts, and interfaces with the electrolyte need to work together to efficiently convert reactants into products. Also the interactions between the reactants with the catalytic surface of the (photo)electrocatalytic device is of primary importance to determine the selectivity and activity of the device. Unfortunately, these devices are often unstable or exhibit insufficient activity or selectivity for the CO2 reduction reaction (CO2RR). In addition to the thermodynamic requirements, the semiconductor/electrolyte interface also plays a crucial role in determining the performance of photoelectrodes, directly influencing the efficiency and performance of artificial photosynthetic systems.

In this context, we present a few examples of how light-absorbing materials can be utilized in integrated photoelectrochemical cells or when directly interfaced with the electrolyte for HER and CO2RR. The talk will span metal oxide materials, silicon, and halide perovskites. We will also look into modification of the local catalytic environment to address selectivity issue in photoelectrochemical CO2 reduction. Our approach demonstrates that the mechanistic understanding of these complex systems can lead to improved stability and performance of various photoelectrode materials used in these reactions.

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Bringing living photovoltaics to life with nanobioengineering

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Solar-driven biocatalysis for unlocking a sustainable future

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Inspired by the molecular principles of natural photosynthesis, solar conversion technologies have emerged as a promising solution to provide not just green electricity, but also energy vectors in the form of solar fuels and chemicals. Directions taken include synthetic, biomolecular or biohybrid photoelectrochemical devices characterised by various degrees of integration. Implementation of the synthetic artificial photosynthetic systems (APS) is hampered by the necessity to apply harsh conditions for the catalysis in each half-cell to occur efficiently, photocorrosion of electrode materials, often-limited product selectivity and catalyst instability. Moreover, the best performing inorganic and molecular catalysts usually encompass rare/toxic elements, which precludes such APS systems from large-scale implementation. Therefore, the alternative field of biomolecular and biohybrid artificial photosynthesis has emerged by combining the biotic components, which have been evolutionary optimised in their photocatalytic performance, with non-toxic and cost-effective synthetic materials for selective production of target chemicals at ambient conditions.

In this lecture, I present the bottom-up rational design that can yield the increased solar conversion efficiency and stability in biomolecular systems based on the robust photoenzyme, photosystem I (PSI). The PSI biophotocatalyst in these devices is interfaced with various cost-efficient, transparent electrode materials for production of green electricity and fuel. I will show that the performance of PSI-based devices is 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 molecular interface significantly improves not only the light-harvesting functionality of the PSI photoenzyme but also increases its long-term photochemical stability and the overall photoconversion efficiency. Such rational design paves the way for generation of viable and sustainable biomolecular technologies for solar energy conversion into fuel and carbon-neutral chemicals.

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Molecular engineering of the abiotic/biotic interface for efficient solar-converting biophotovoltaics

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Solar-converting nanosystems using biomaterial resources carry great potential for developing sustainable technologies to ameliorate climate change and minimize reliance on fossil fuels. By mimicking natural photosynthesis, diverse proof-of-concept biosolar systems have been used to produce green electricity, fuels and chemicals. Although increasingly efficient devices have been reported, notably those with 3D nanostructured electrodes for improved biocatalyst loading and light absorption, the best-performing systems keep employing freely-diffusing toxic mediators and mass transfer processes, precluding a large-scale implementation. To overcome these limitations, a key factor is to ensure efficient electronic communication between biocatalysts and the electrode together with the appropriate orientation of (photo)electroactive protein to achieve the highest possible charge transfer efficiency and minimize wasteful back reactions.

Here, we present a strategy developed in our laboratory to optimize the abiotic/biotic interface by rationally engineering a covalent molecular interface. The metalorganic interface, compatible with various transparent conducting oxide (TCO) and graphenoids, is terminated with nitrilotriacetic acid (NTA) metal complexes. This universal molecular anchors serves to immobilize in an oriented manner His_6-tagged proteins, such as biophotocatalysts and other redox-active proteins. The photoelectrochemical properties of the modified TCOs shown that the covalent functionalization induces a p-doping of the electron-rich surfaces, resulting in an enhanced unidirectional cathodic photocurrent up to 1 $\mu A \cdot cm^{-2}$.

The engineered interface was furher employed for the construction of biophotovoltaic devices incorporating His_6-tagged cytochrome c (a natural electron relay) and photosystem I. In the resulting biophotocathodes, we identified a controllable effect at the abiotic/biotic interfaces essential for achieving more effective vectorial electron transfer. The (bio)nanoarchitectures with a boosted interface depict much-higher photocurrent outputs and faster electron transfer kinetics compared to the unboosted analogs, even in presence of freely-diffusive mediators. These results open a new avenue for efficiently interfacing biomachineries, providing a benchmark design advancement in the quest for viable biohybrid technologies.

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Balancing the harms and benefits of sun exposure

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Exposing the skin to the harm has both harms and benefits. It is the main cause of skin cancer and premalignant lesions, but it also leads to the production of vitamin D and other benefits are emerging. The wavelengths that are most directly linked with skin cancer are the same as those that produce vitamin D, so finding the balance is challenging. Importantly, the balance of benefits and harms is not the same for all people. In light of this, a new position statement has been released which explicitly provides advice that recognizes the diversity of Australia's population. People with skin that is very sun sensitive should avoid deliberately exposing the skin to the sun, and should be vigilant with sun protection and use vitamin D supplements to meet their vitamin D requirements. Those with deeply pigmented skin do not require routine sun protection, and more time outdoors is needed to avoid vitamin D deficiency. The third group, at intermediate risk, can aim to obtain a vitamin D-effective dose of UV radiation on most days of the week, but sun protection remains extremely important. Sunscreen is a pivotal part of sun protection. However new evidence suggests that routine sunscreen application can lead to increased risk of vitamin D deficiency, suggesting that vitamin D supplementation might be required for people who adhere to this recommendation. Details underpinning the new position statement, and the new sunscreen trial results will be presented.

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Ironing out skin photoaging with multifunctional natural-based products with potent iron chelating and antioxidant properties

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Excessive exposure to ultraviolet (UV) radiation from the sun remains the primary cause of skin photoaging and cancer. While sunscreen use is widely recommended for protecting the skin, empirical evidence regarding the unequivocal efficacy of current sunscreens is scarce. Consequently, it is crucial to re-evaluate the effectiveness of existing sunscreens in safeguarding against sun exposure. Moreover, regulatory scrutiny has intensified due to the demand for safe and efficient sunscreens. Notably, the effectiveness of current sunscreens against the UVA component of sunlight appears limited. UVA, with its dual damaging effects, interacts with intracellular chromophores, generating reactive oxygen species (ROS). These ROS directly harm skin components, leading to photodamage and photoaging. Additionally, UVA-induced ROS triggers an immediate increase in reactive "free iron" within cells, exacerbating oxidative damage and further sensitising the skin cells to subsequent UVA exposure. This talk will provide an overview of novel strategies used in our laboratory to design bio-inspired multifunctional sunscreen ingredients and UVA photo-protectants with both antioxidant and/or iron chelating properties to tackle the dual damaging effects of UVA component sunlight against skin photodamage and photoaging.

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cNOS: A Key Regulator of Redox Homeostasis and DNA-damage Repair in Skin Cells Post-UV Exposure

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Ultraviolet (UV) radiation triggers a rapid increase in nitric oxide ($NO^{\cdot}$) production by constitutive nitric oxide synthases (cNOS), which disrupts cellular redox homeostasis. This surge in $NO^{\cdot}$ has a complex relationship with DNA damage and repair in skin cells. Upon UV exposure, cNOS is activated and produces NO·, which readily reacts with superoxide ($O_2^{\cdot-}$) to form peroxynitrite ($ONOO^{-}$), a potent inducer of cyclobutane pyrimidine dimers (CPDs) - a hallmark of UV-induced DNA damage. Our study suggests cNOS also plays a critical role in DNA repair mechanisms post-UV. To unravel this paradox, we investigated the influence of cNOS on DNA damage and repair using cultured skin cells, skin explants, and SKH-1 mouse models. We observed that inhibiting cNOS activity indeed reduced markers of UV-induced DNA damage like γH2AX and CPD formation. However, this seemingly beneficial effect was countered by a concurrent impairment in DNA repair capacity after UV exposure in cNOS-deficient models as cNOS knockout leads to a slight increase of CPD inmediately after UV exposure which persists over the time. These findings suggest a dual role for cNOS: on one hand, it can contribute to DNA damage through $NO^{\cdot}$ and $ONOO^{-}$ production, while on the other hand, it appears to be essential for efficient DNA repair mechanisms following UV irradiation.

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Oxidative modifications of melanin pigments increase their photosensitizing ability

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Melanin, particularly eumelanin, is considered an efficient photoprotective pigment, even though its residual photochemistry is responsible for the generation of reactive oxygen species (ROS). However, the flux of ROS, photogenerated by melanin under typical conditions, is too low to cause significant disruption of pigmented cells. The phototoxic potential of melanin could substantially increase with an enhanced photosensitizing ability of the melanin. We have tested this postulate by analyzing the photochemical and photobiological properties of human hair melanosomes obtained from individuals of different skin phototypes

subjected to different degrees of experimental photobleaching [Mokrzynski et al., 2023]. Melanosomes of skin phototypes II and III were most susceptible to bleaching induced by intense violet light. Upon excitation with UVA or blue light, melanin from control melanosomes of phototype II and III exhibited the highest yield to photogenerate singlet oxygen. Experimental bleaching of hair melanosomes modified their photochemical properties. Thus bleached melanosomes of all tested skin phototypes revealed higher efficiency to photogenerate singlet oxygen. On the other hand, the ability of bleached melanosomes, except melanosomes from skin phototype V, to photogenerate superoxide anion, was lower than that of control unbleached melanosomes. The antioxidant capacity of hair melanosomes was consistently reduced by photobleaching. Photobleached melanosomes of all skin phototypes, except phototype V, were phototoxic to human epidermal keratinocytes (HaCaT cell line). Irradiation of such cells, containing photobleached melanosomes, with light from a solar simulator, decreased the cell's mitochondrial membrane potential and increased the cellular level of lipid hydroperoxides. The data suggest that photoaging of melanin could substantially elevate its photosensitizing ability.

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Visible light excites lipofuscin and induces photoaging in skin cells

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Lipofuscin is a yellow-brown cross-linked granular material, which is resistant to enzymatic hydrolysis and accumulates in various tissues, as a result of the normal aging process, consequently, it has been called the aging pigment. It is composed of highly oxidized proteins (30-70%), lipids (20-50%), and carbohydrates (4-7%) (1). Lipofuscinogenisis has been linked to the failure in the homeostasis of the skin redoxome, favoring the accumulation of reactive electrophiles in lysosomes, such as 4-hydroxy- 2-nonenal (HNE), which favors the crosslink of the oxidized molecules(2). The chelating properties of lipofuscin also favors the incorporation of metal cations (up to 2%), especially iron. Excitation of lipofuscin with UVA, blue, or green light results in yellow/blue fluorescence. After light absorption, lipofuscin also act as a potent photosensitizer, generating excited states such as triplet excited states and singlet oxygen ($^1O_2$) as well as a variety of radicals, accelerating the photodamage on the pigment´s vicinity (3). Lipofuscin-like granules have been identified in keratinocytes exposed to UVA, turning these cells hyper-sensitive to broad spectra Visible Light (VL) and causing exposed cells to accumulate mutagenic DNA lesions (4). The damage in nuclear DNA is correlated with the accumulation of $^1O_2$ – induced 8-oxo-dG and other oxidation products, as well as single and double-strand breaks. The phototoxicity action spectra of VL in keratinocytes demonstrated that the mutagenic potential of violet (408 nm) and blue (466 nm) light is much larger than those induced by green and red light. The violet/blue component of VL behaves similarly to UVA in keratinocytes, producing fpg-sensitive mutagenic lesions and inhibiting the autophagic flux (5). In conclusion, lipofuscin accumulation can occur in human skin as a consequence of sun bathing with classical sun screen, i.e., those that do not offer broad-band (specially efficient UVA and blue light) photoprotection. Public and health professionals should be aware that the excess of visible light exposure can have nasty consequences to the human skin.

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Photosensitization of FICZ and ICZ in mimetic models of membranes

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Endogenous photosensitizers (EP) are responsive to solar photon absorption, leading to oxidative stress upon prolonged sun exposure. Among these, 6-formylindole [3,2-b] carbazole (FICZ) (fig.1A) stands out for its potent properties as an aryl hydrocarbon receptor (AhR) agonist[1], but it's role in photooxidative stress remains incompletely understood[2]. To elucidate the impact on cellular lipids, we examined the photophysical and photochemical characteristics of FICZ and compared them with indole [3,2-b] carbazole (ICZ), a non-typical EP lacking triplet species and singlet oxygen formation. Our objective was to investigate FICZ's oxidative stress on giant unilamellar vesicles (GUVs). To investigate the role of FICZ on oxidative stress, GUVs were prepared via the natural swelling method with EP and DOPC, and then examined using epifluorescence microscopy. Previous photochemical and photophysical assays showed that FICZ has ΦΔ of 0.53 and Φf of 0.15, contrasting ICZ with ΦΔ of 0.05 and Φf of 0.30.[3] In HaCaT cells, FICZ induced phototoxicity and AhR translocation, while GUVs photosensitized with FICZ exhibited hydroperoxide formation (fig.1C), consistent with its ΦΔ, whereas ICZ led to instability due to its rigid structure.

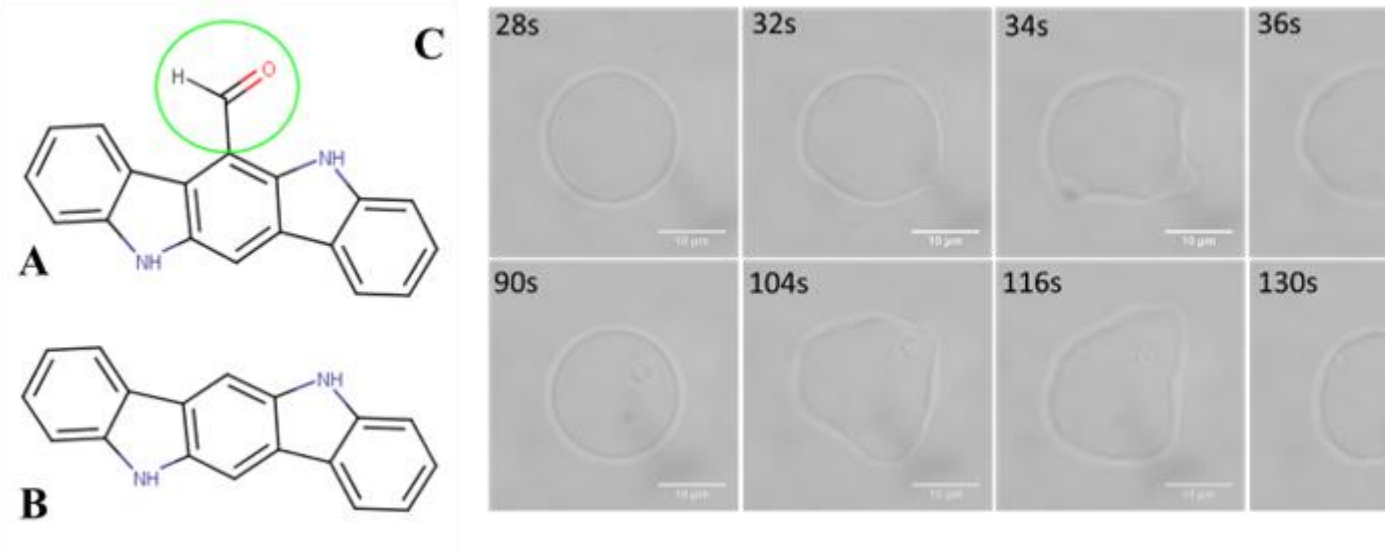

Figure 1. Molecular structure of FICZ (A) and ICZ (B). GUV of FICZ:DOPC (1:100) observed over 158 seconds under UVA irradiation. Scale 10µm.

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Hyperpigmented disorders, role of sunlight and photoprotection needs

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Abstract content TBC

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Beyond sunscreens: Oral and systemic photoprotetion

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While the strategy for comprehensive photoprotection of seeking shade, wearing photoprotective clothing, wide brimmed hat and sunglasses, and applying sunscreen to otherwise exposed sites is well-established, oral and systemic photoprotection has been studied for many years. For example, down regulation of UV-induced cutaneous changes has been described for *Polypodium leukotomos* since the mid 1990s, and for green tea since at least 2003. In 2015, a phase 3 randomized trial of nicotinamide for skin-cancer chemoprevention was published, and the effective of subcutaneous implant of afamelanotide in patients with erythropoietic protoporphyria (EPP) was reported.

Compared to topical measures and behavioral modification, the advantage of oral photoprotection is its convenience. However, currently, all the oral photoprotective agents should be used as adjunctive measures to the established photoprotection strategy. The sole exception is afamelanotide, which was approved by US FDA for EPP as subcutaneous implant on Oct 8, 2019.

As all the agents (except for afamelanotide) are over the counter preparation, judicious use might be helpful to partially down-regulate the side effects of sun exposure.

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New insights in visible light-induced pigmentation and means of protection

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Hyperpigmentation and pigmentary disorders are major consequences of sun exposure. The role of all UV rays up to the longest UVAs is now well established. In addition, Visible Light (VL) also contributes to the development, aggravation, or relapses of pigmentary problems such as dyschromia, melasma or post-inflammatory hyperpigmentation.

In the VL wavelengths' domain, the shortest wavelengths are the most efficient in inducing pigmentation[1,2], especially HEV (High Energy Visible Light 400-450nm) and Blue light (400-500nm) contributing for 47 and 71 % in VL-induced pigmentation. This pigmentation is mostly observed in individuals Fitzpatrick Phototype III and above. Within these populations, the level of induced-pigmentation was compared after four exposures to 144J/cm² of VL. In three groups, European FPTIII, African descent FPTVI and Asian descent FPTIII individuals, significant differences were observed; the highly pigmented, FSPVI as well as Asian FSPIII subjects having a higher response to VL compared to Europeans FSPIII.

To provide VL photoprotection, the efficient solutions relies on the use of pigments. Robust in vivo method can determine a VL-PF (Visible Light Protection Factor) which can be expressed as a percentage i.e. pVL-PF ranging from 0 to 100% performance[3]. Using a similar methodology, 30 different formulas were assessed. We propose a new framework to calculate pVL-PF together with statistical indicators allowing to rank formulas' efficacy. In parallel the VL transmittance profile of the formulas showed a very high correlation with in vivo performance. These data provide methodologies to quantify VL photoprotection.

In conclusion, photoprotection should take into account all UV rays, but also VL, especially for populations with a high susceptibility to develop pigmentary disorders.

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A new sunscreen filter protects against pigmentation, and molecular damage *in vivo* and *in vitro* at the UVA/visible boundary region

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Skin pigmentation by solar ultraviolet radiation (UVR; ~295–400 nm) is well established. More recently, visible light (VL; 400–740 nm) has been shown to induce rapid pigmentation. Such pigmentation is thought to be caused by oxidative stress, which has associations with skin cancer and photoageing. However, the UVR-VL boundary region has been less well studied. The lower back of healthy Fitzpatrick skin type (FST) II-IV individuals was irradiated with increasing doses of narrow-band 385 nm and 405 nm radiation [1]. Pigmentation change was measured immediately, 6 h and 24 h post-irradiation using two reflectance spectroscopy devices and visual grading. Pigmentation was dose-dependently increased in all skin types and time points for both spectra. Two sunscreens, both labelled SPF 15 and UVA protective in the EU and USA (but with different Boots star rating in the UK, 2* *vs* 5*) were compared. Their formulations were identical apart from the addition of a new organic filter bis-(diethylaminohydroxybenzoyl benzoyl) piperazine (BDBP) that absorbs between 350 and 425 nm. The product that lacked BDBP provided minimal protection against pigmentation, but its addition provided almost complete protection. This demonstrates the needs to improve photoprotection at the UVR-visible border and for sunscreens to act as neutral density filters.

The same sunscreens were also assessed for their ability to prevent oxidative stress, gene expression for photoageing, inflammation and oxidative stress, DNA damage ("dark" cyclobutane pyrimidine dimers) in HaCaT keratinocytes and *in vivo* in healthy FST I-II volunteers. The formulation including the new filter provided significantly more protection than the conventional sunscreen for almost all endpoints tested. This demonstrates the requirement for improved photoprotection at the UVR-visible border region.

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Photosensitization as a Tool for addressing Drug Resistance

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Drug resistance is a multifactorial phenomenon studied in detail in microbiology where it evolves rapidly because of genetic promiscuity in bacteria. However, it is also a major problem in cancer therapeutics and shares some commonalities with our microbe relatives. Multidrug resistance (MDR) is a term used commonly in both fields and refers to several mechanisms of resistance. One of the primary ones is the development of efflux pumps that pump out or bind to therapeutics preventing them from acting at target sites. The development of antiapoptotic protein overexpression or DNA repair mechanisms are only some of the other mechanisms. Other mechanisms involve the development of preventing drug delivery or suppression of the immune response. This presentation will focus on how photodynamic activation can be used as a tool to overcome some of these pathways of drug resistance acquisition/induction and how it might be a unique tool in the armamentarium of combination cancer therapeutics.

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Can cancer cells escape photodynamic therapy?

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For more than two decades, we have studied the unique characteristics of cells resistant to Photodynamic Therapy (PDT) following repeated *in vitro* treatment sessions. Among the many PDT-resistant cells isolated in our laboratory, we have never found cross-resistance to other photosensitizers (PS) or chemotherapeutic drugs, suggesting the involvement of PS-specific factors rather than general antioxidant defense systems.

Developing a significant level of resistance requires multiple PDT rounds. For example, 10 to 15 cycles of aminolevulinic acid (ALA)-PDT led to a 4 to 7-fold increase in resistance in LM3 murine mammary tumour cells [1], while human mammary Ras-transfected HB4a cells exhibited slight resistance (1.2 to 2-fold) after three successive PDT cycles using different PSs: ALA, Verteporfin, m-THPC, and Merocyanine [2]. However, complete cell killing was achieved by increasing the light dose.

All PDT-resistant cell populations we isolated were less migratory and invasive than their parental counterparts and demonstrated significantly impaired metastatic potential in vivo. Additionally, these cells showed decreased general anchorage-dependent adhesion and invasion, along with disrupted cytoskeletons and altered expression of adhesion proteins.

Interestingly, we have also found that even when Ras-transfected cells present lower adherence to extracellular matrix proteins, this does not make them more sensitive to PDT or chemotherapy. On the contrary, a marked gain of resistance to PDT was observed in floating cells compared to adhesive cells, accounting for the higher ability conferred by Ras to survive in conditions of decreased cell-extracellular matrix interactions [3].

The presence of cancer stem cells (CSCs) has recently been considered a major cause of failure in anticancer therapies. While the current state of the art suggests that successful elimination of CSCs can be achieved after PDT, our studies indicate an enrichment of the stem cell fraction in IGROV-1 ovarian cancer cells after exposure to PDT selective pressure, evidenced by higher OCT4 and NANOG expression and a higher number of sphere formation.

Recently, we found that SKOV-3 and IGROV-1 ovarian cancer cells resistant to cisplatin did not exhibit cross-resistance to PDT. Conversely, PDT-resistant ovarian cancer cells remained responsive to cisplatin and paclitaxel treatments and were equally migratory and invasive compared to the parental cell lines.

The dual action of the photosensitizer and light in PDT, along with the multiple sites of action targeted, makes the development of resistance quite unusual in a clinical setting.

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Resistance in non-melanoma skin cancer: how to overcome it

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Non-melanoma skin cancer (NMSC) is the most common type of cancer worldwide. Treatment options for NMSC include Photodynamic therapy (PDT). PDT is used mainly for small and superficial tumors, including actinic keratosis, in situ squamous cell carcinoma and basal cell carcinoma. The principal compounds used in PDT for NMSC are ALA and MAL, precursors of the photoactive compound protoporphyrin IX (PpIX). PDT produces very satisfactory results in clinic; however, some cells can survive, producing tumor relapses and/or increases in their clinical and biologic aggressiveness. Even intrinsic cell tumor factors (specific mutations), tumor microenvironment, constituted by cancer cells, immune and endothelial cells, extracellular matrix fibers and particularly fibroblasts (cancer-associated fibroblasts, CAFs), play a crucial role in modulation the activity, growth, and the resistance to cancer therapies. Therefore, our objective is to define factors implicated in the response to PDT with the goal of improving PDT by itself or by combining it with other treatment modalities to target both, cancer cells and CAFs, to eradicate the tumor lesion. In this sense, we have evaluated the combination of PDT with 5-FU, metformin and rapamycin in bidimensional and tridimensional in vitro models (spheroids) as well as in xenografts.

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Using PCI to overcome PDT resistance mechanisms in cancer

Pål Kristian Selbo[2, 1], Sabine Weisheit[2, 1], Catherine Sem Wegner[2, 1], Ieva Ailte[2, 1], Maja Radulovic[2, 1], Anette Weyergang[2, 1], Kristian Berg[2, 1], Andreas Brech[2, 1]

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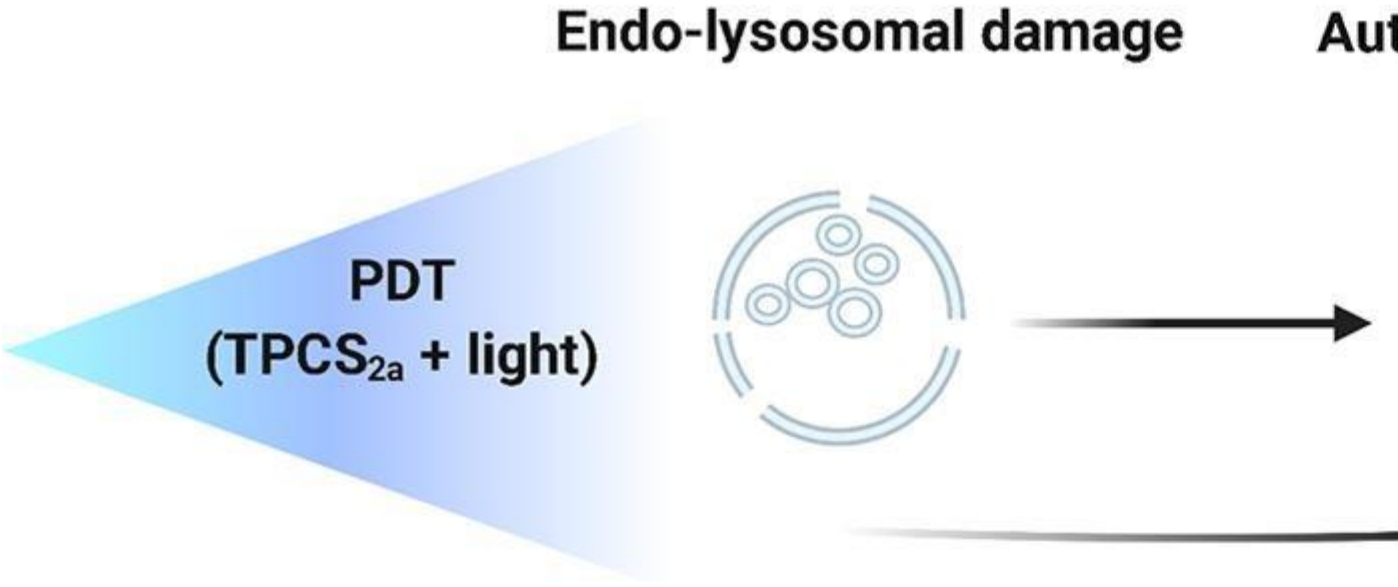
In advanced-stage cancer, autophagy plays a crucial role in tumour cell survival, making it a potential target for cancer treatment. Photodynamic therapy (PDT) can induce autophagy, leading to cancer cell death when apoptosis is compromised. Conversely, at low-dose PDT, autophagy serves as a protective mechanism in cancer cells, allowing them to repair damage caused by oxidative stress. In this presentation, I will discuss cellular resistance mechanisms against PDT, focusing on autophagy. Our

previous studies demonstrated that PDT using photochemical internalization (PCI)-photosensitizers localized in lysosomal membranes directly damages both lysosomes and the key autophagy regulator mTOR. Recently, we found that low-dose PDT with the PCI photosensitizer TPCS2a (also known as fimaporfin or Amphinex) induces autophagy while at high-dose PDT inhibits autophagic flux. After TPCS2a-PDT, the activation of the 4EBP1 protein (a substrate of mTOR) significantly decreased in PDT-treated cells compared to controls. The autophagic response post low-dose TPCS2a-PDT involves recruitment of ubiquitin (Ubq), autophagic adaptor protein p62, and microtubule-associated protein 1A/1B-light chain 3 (LC3) to damaged vesicles, marked by Galectin 3 (Gal3). Ultrastructural analysis revealed a thick p62-positive layer surrounding these permeabilized vesicles. Although p62 appears crucial during selective autophagic sequestration, its presence isn't essential for effective removal of damaged vesicles or lysosomal content recovery. Interestingly, an active autophagic response and p62 presence are vital for cancer cells to survive low-dose TPCS2a-PDT. Thus, targeting of p62 may constitute a rational strategy to improve the cytotoxic efficacy of PDT. Hence, to overcome PDT resistance, we can either inhibit autophagic flux by targeting lysosomes and/or directly address proteins crucial for autophagy and cell survival. Alternatively, we propose using PCI to disrupt endosomes and lysosomes, releasing entrapped cancer drugs into cancer cell cytosol, where autophagy provides strong cytoprotection.



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Advancing Glioma Immunotherapy: Photodynamic Therapy-Induced Immunogenic Cell Death and Dendritic Cell Vaccines

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Gliomas, the most frequent type of primary tumor of the central nervous system in adults, results in significant morbidity and mortality. Despite the development of novel, complex, multidisciplinary, and targeted therapies, glioma therapy has not progressed much over the last decades. Therefore, there is an urgent need to develop novel patient-adjusted immunotherapies that actively stimulate antitumor T cells, generate long-term memory, and result in significant clinical benefits.

Immunogenic cell death (ICD) plays a pivotal role in triggering immune responses essential for effective anti-cancer therapies. A critical aspect of ICD is achieving a balanced combination of adjuvanticity and antigenicity. Adjuvanticity involves the release of damage-associated molecular patterns (DAMPs) primarily derived from dying cancer cells. These DAMPs, along with cytokines and chemokines, serve as adjuvants facilitating the recruitment and maturation of antigen-presenting cells. However, the presence of these adjuvant DAMPs signals alone is not sufficient to elicit an effective immune response against cancer cells. The cancer cells must also possess strong antigenic properties. Antigenicity is mediated by tumor-associated antigens predominantly presented by dendritic cells, particularly by the generation of neo-epitopes. Many anticancer agents and strategies induce ICD, but despite their robust effects *in vitro* and *in vivo* on mice, translation into the clinic remains challenging.

Therefore, in this work the therapeutic efficacy and molecular mechanisms responsible for the generation of anti-tumor immunity generated by dendritic cell (DC) vaccines loaded with ICD glioma lysates have been investigated. ICD has been induced by photosens-based photodynamic therapy. Here, I will first discuss the main principles of ICD, and then I will discuss the intriguing results obtained on orthotopic intracranial vaccination glioma mouse models. This work demonstrates the therapeutic feasibility of using DC vaccines loaded with glioma cells undergoing ICD and will open promising avenues for the development of novel immunotherapy for glioma.

Acknowledgments

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Repairing a sunburn: flipping the skin-immune switch

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This talk will discuss immune modulation as a strategy for repairing the skin after a severe UV sunburn. Our group has had a long interest in investigating treatments to repair the skin following injury from sunburns and from exposures to toxic chemicals and radiation. We have shown that high dose oral vitamin D3 mitigates inflammation from experimental UV-induced sunburns in randomized double-blinded placebo-controlled human clinical studies. Subjects receiving vitamin D3 had reduced expression of pro-inflammatory mediators TNFa and iNOS in the skin 48 hours after a sunburn. Clustering subjects based on global gene expression profiles shows that those with higher serum vitamin D3 levels after intervention had increased skin expression of arginase-1 (Arg-1), a pro-resolution gene expressed by reparative macrophages. Modeling these studies in animal models, we have shown that skin recovery from sunburns is mediated by the action of anti-inflammatory M2 macrophages to promote epidermal regeneration. Furthermore, we demonstrate that vitamin D enhances macrophage autophagy and polarization towards the M2 repair phenotype. Pharmacological inhibition of autophagy increased UV-induced apoptosis and inhibited M2 recruitment into the skin resulting in severe skin damage. More recently, we demonstrate that a novel topical synthetic melanin working as a potent ROS scavenger augments the skin microenvironment facilitating recruitment of reparative TGF-b+/IL-10+ monocytes and M2-macrophages to heal the skin.

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More than skin-deep: exploring the immunomodulatory effects of ultraviolet radiation

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The ultraviolet radiation (UV) in sunlight can suppress the immune system, which explains why sunlight is such a powerful carcinogen. At the same time, sunlight exposure can protect against some inflammatory diseases, such as multiple sclerosis (MS). This is demonstrated by the latitude-gradient effect, where geographical regions with low UV exposure are associated with higher MS prevalence. Dermatologists have used artificial UV light to control skin inflammation for decades. It is not clear whether UV phototherapy can produce the immunological effects that afford protection against systemic (non-skin) inflammatory diseases. We investigated the immunomodulatory capacity of narrowband UVB (NBUVB), which contains a narrow range of UV (311–312 nm) and is commonly used to treat psoriasis. We examined immunological effects of NBUVB in mice both at the site of irradiation (skin) and in distant tissues (blood, lymph node and spleen). We found that while NBUVB induced some of the local cellular changes characteristic of sunlight exposure (neutrophil infiltration and Langerhans cell depletion), mast cells were surprisingly unaffected. Sunlight can cause systemic immune suppression by disrupting the normal movement of lymphocytes around the body, but NBUVB did not activate this pathway. However, NBUVB effectively suppressed antigen-specific T cell-mediated killing in the spleen. In summary, NBUVB activated some, but not all of the immune-suppressive pathways associated with sunlight exposure. Understanding the wavelength-dependant effects of UV on the immune system will allow us to more effectively harness its immunomodulatory capacity to treat a wider range of diseases.

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Seasonal sunlight exposure (daylength and UV) is associated with regulatory T-cell and Th17 levels in adolescent and adult females, a potential risk factor for MS

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The higher prevalence of multiple sclerosis at higher latitudes is associated with reduced sunlight during childhood, and female sex. Alterations in inflammatory Th17 and regulatory T-cells are associated with immune auto-reactivity, with regulatory T-cells being important in suppressing reaction against self tissue. In Hobart, Australia (latitude 42.8^0 south), thirteen girls (aged 12-13) and fifteen mothers (aged 34-55) had blood collected in the evening in daylight in February/March, (prior daylight 13-15 hours) and at the same time in the dark in August/September (9-11 hours daylight). Height and weight were measured. Participants completed online surveys prior around menstruation, sleep, exercise and time outside. Proportions of Th17 (CD4+, CXCR3-, CCR4+, CCR6+, CD161+), total Treg (CD4+, CD25+, CD127low), naïve Treg (CD45RA+, CD4+, CD25+, CD127low) and memory Treg (CD45RA low, CD4+, CD25+, CD127low), relative to CD4+ T-cells, were enumerated by flow cytometry (Cytek Aurora).

Hours spent outside were greater in summer than winter (12.5 v 10.5, p=0.0003), the %Treg was higher in summer than winter (7.3% vs 7%, p=0.002), including memory Treg (3.1% vs 2.9%, p=0.01), and naïve Treg (4.2% vs 4.0%, p=0.03), whereas %Th17

remained unchanged. In women, a negative correlation between the number of hours spent outside in summer and %Th17 was observed (r=-0.53, p=0.035). The %Th17 cells was higher in women than girls (4.9% vs 3.1%, p=0.001) whereas girls had a higher total %Treg (7.6% vs 6.7%, p=0.005), consisting of a greater % naïve Treg (5.5% vs 3.7%, p=0.0001) whereas the women had a higher %memory Treg (3.4% vs 2.6%, p=0.0001). These light dependent seasonal differences may influence immune development in adolescents and future auto-reactivity.

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The role of UV-induced regulatory T cells in the establishment of Cutaneous Squamous Cell Carcinoma

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Cutaneous squamous cell carcinoma (cSCC) is one of the most prevalent cancers in Caucasian populations, and its aggressive form poses a high risk of metastasis resulting in increased rates of mortality and morbidity. Recurrent and chronic exposure to ultraviolet (UV) radiation from the sun plays a crucial role in the initiation, development, and perpetuation of cSCC. Accumulating evidence suggests that regulatory T (Treg) cells are associated with UV-induced immunosuppression, however, a direct role for these cells in the establishment of cSCC remains elusive. Following UVB exposure, $CD4^+CD25^+$ Treg numbers were found to increase significantly in the skin-draining lymph nodes of treated mice. Mice that had been exposed to UVB showed reduced ear swelling in response to ovalbumin challenge in a contact hypersensitivity assay. Reduced ear swelling responses were lost when Tregs were depleted with anti-CTLA-4, anti-TIGIT or anti-FR4 antibodies, suggesting that UV-induced Tregs were functionally suppressive. Following the treatment of mice with UVB for different lengths of time (2w, 4w, 6w, 8w), it was determined that eight weeks of UVB treatment consistently allowed the establishment and growth of adoptively transferred cSCC tumour fragments from donor mice. We aim to target Tregs in these tumour models to determine whether Treg depletion or manipulation reverses the capacity of UVB to enable cSCC tumour establishment. Overall, this study examines the plausibility of Treg manipulation as a preventative strategy to prevent UV-induced cSCC tumour establishment.

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State Transition in Green Algae : Structural Dynamics and Evolutionary Perspectives

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In green algae and plants, state transitions act as a short-term acclimation process that balances excitation between PSI and PSII. During this process, LHC trimers are phosphorylated, dissociated from PSII, and reassociated with PSI. We determined the Cryo-EM structure of the PSI supercomplex from the green alga *Chlamydomonas reinhardtii* in state 2, revealing the structural details of the binding site of the phospho-LHCII trimers to the PSI–LHCI supercomplex (1). A subsequent study elucidated the Cryo-EM structure of the PSI supercomplex from the low-light-grown prasinophytic alga *Ostreococcus tauri*, which is ubiquitously present in the ocean. This structure exhibits a unique composition, involving a phospho-Lhcp trimer bound to the PSI core along with two additional Lhcp trimers, suggesting the possibility of state transition capability in this early-branching green alga (2). To investigate this hypothesis, we conducted a series of biochemical and physiological experiments. Initially, the absorption spectra showed a distinct difference between PSI and PSII, particularly at blue-green wavelengths. Subsequently, we observed that specific excitation of Lhcp with green light induced its phosphorylation and led to the formation of the PSI-LHCI-Lhcp supercomplex. Furthermore, the functional antenna size of PSI could reversibly expand in response to green light/darkness, demonstrating that *O. tauri* undergoes state transitions. These findings not only highlight a unique photoacclimation to the marine environment performed by *O. tauri* but also suggest an ancestral role of state transitions in green plants, considering the phylogenetic position of prasinophytes.

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2. 2. Ishii, A. et al. (2023) eLife 12: e8448

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A dynamic pair: Photosystem I and the cytochrome b_6f complex in focus

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Electron transfer between the major complexes of the photosynthetic apparatus is mediated by small electron transfer proteins. In order to fulfill their role, these proteins have to form interactions which are on one hand very specific, but simultaneously match multiple target complexes. This trait triggered a synergized evolutionary path, in which a change in one complex initiated adequate adaptations in matching residues of other complexes. In this study, we examined plastocyanin (PC) binding and electron transfer with both photosystem I (PSI) and cytochrome b_6f (cyt b_6f), and show the synergetic adaptations between these three enzymes. Furthermore, we explored the effects of PC phosphorylation on these interactions. To do so, we generated several recombinant variants of PC, in which we genetically engineered two of the phosphorylated residues (S10 & S49). We studied the kinetics of both Cyt*f* oxidation and P700 re-reduction by measuring fast optical spectroscopy. We also conducted chemical protein crosslinking and structural proteomics to gain further insights on the interaction between PC and cyt b_6f. Our results show that the phosphorylation mode of PC alters the conformation in which they establish binding and electron transfer, and generated new models which elaborate the mechanism of this adaptation. To further address electron transfer into cyt b_6f via PSI reduced

ferredoxin, we generated site-directed mutants in the N-terminal domain of cyt b_6f subunit IV by chloroplast transformation. These mutations impact state transitions, electron transfer within and into cyt b_6f as revealed by proteomics, fluorescence and fast optical spectroscopy.

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Rewiring photosynthetic electron transfer using CRISPR-Cas9 gene editing

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Fixing CO_2 via photosynthesis requires ATP and NADPH. Linear electron transfer (LET) supplies both metabolites, yet depending on environmental conditions, additional ATP is required which can be generated by cyclic electron transfer (CET). How the balance between LET and CET is set remains largely unknown. Ferredoxin (Fd)-$NADP^+$ reductase (FNR) has been suggested to act as the switch, channelling photosynthetic electrons to LET when it is bound to photosystem I (PSI) or CET when bound to cytochrome b_6f. Testing this hypothesis by direct FNR gene knock-out is prevented by its essential role in LET. Here, using CRISPR-Cas9 gene editing in *Chlamydomonas reinhardtii,* we circumvented this to create cells expressing a chimeric form of FNR tethered to PSI via PSAF. The results provide fascinating and unexpected insights into the role of FNR in regulating photosynthetic electron transfer.

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Photosynthetic light-harvesting regulation utilizing protein dynamics

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Photosynthetic organisms have achieved sophisticated photochemical reaction systems consisting of pigment-protein complexes. The light-harvesting antenna complex (LHC) absorbs sunlight and transfers the light energy to the reaction center (RC), where charge separation is induced. A series of light reactions occurs among pigment molecules embedded in the complexes. The molecular arrangement is highly optimized, leading to an extremely high efficiency of the light reaction. This explains why photosynthesis can continue under dim light conditions. Conversely, under intense light illumination, the system is likely to be photodamaged by an excess light energy. To respond to frequent and significant changes in actual light environments, phototrophs have a variety of photoprotection mechanisms.

The LHC contains multiple pigments, such as chlorophylls (Chls) and carotenoids (Cars). Chls are responsible for the excitation energy transfer via molecular interactions with each other. Conversely, Cars receive energy from Chls and dissipate it as heat, a process known as non-photochemical quenching, thus protecting against excess light energy. The quenching efficiency, which significantly depends on the relative arrangement of Chls and Cars, can be easily perturbed by even slight and local conformational changes around their binding sites in the protein. Considering that protein conformation is thermally fluctuating, the quenching efficiency by Cars should also fluctuate. Thus, to investigate the photoprotection mechanism by analyzing fluctuations in fluorescence properties, we applied single-molecule spectroscopy to the LHC protein.

The fluorescence of individual LHCs exhibited temporal variations in both intensity and lifetime. Statistical analyses of their time sequence data revealed frequent transitions between photoactive and inactive-quenched states, likely reflecting protein conformational fluctuations around the binding sites of Chl and Car. Additionally, under low pH conditions, in which the protein scaffold of LHCs has been reported to vary, the fluctuation behavior was restricted, and consequently, the quenching state was stabilized. The pH drop is induced by the photochemical reaction in the RC, especially under intense light conditions. From these results, it is suggested that LHCs sensitively sense light environmental changes through pH variations and then adjust quenching efficiency by flexibly altering their own structure. In this presentation, I will discuss photosynthetic light-harvesting regulation mechanism associated with protein dynamics.

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Phototherapy of inflammatory skin diseases in 2024

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Abstract content TBC

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Phototherapy of vitiligo

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Vitiligo is characterized by depigmented patches resulting from loss of melanocytes. Vitiligo can be challenging to treat and exhibit an unpredictable clinical course. Phototherapy has emerged as a prominent treatment option for vitiligo, utilizing various light modalities (not only UV, but also visible light) to induce disease stability and repigmentation. Phototherapy is still considered as the “gold standard” treatment for vitiligo, despite the recent introduction of new drugs that target the underlying immune mechanisms involved in vitiligo pathogenesis. Narrowband ultra-violet B, (either conventional NB UVB, 311 nm, or excimer sources 308nm) remains the most commonly employed, studied, and effective phototherapy modality for vitiligo. Recent work

has pointed out the possibility to use a new 311 solid state laser as an alternative to the 308 nm excimer laser. The presentation focuses on the clinical applications and molecular mechanisms of phototherapy in vitiligo. Special attention is given to reviewing different types of lamps, guidelines, and the utilization of targeted phototherapy modalities. Additionally, the integration of phototherapy with other treatment modalities, including its use with the new drugs (JAK inhibitors) in vitiligo, is discussed. The issues represented by photo-adaptation and the possible risk of carcinogenesis in human skin are also examined. Also, red-light and blue light therapy via LEDs can stimulate repigmentation in patients with vitiligo with minimal adverse events. Recently the use of heliotherapy has been reappraised when therapy with artificial UV is not available: exposure to natural sunlight can be monitored with a dedicated App in order to help the patients to select appropriate location and duration of exposure based on real time dosimetry. The review underscores the evolving landscape of phototherapy and offers insights into optimizing therapeutic outcomes and addressing the challenges ahead. Phototherapy is a valuable therapeutic option for managing vitiligo, with potential for further advancements.

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Research progress and trends in laser treatment of acne scars:a bibliometric analysis of related research over the period of 2014-2023

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Background : The management of acne scars poses a significant challenge for dermatologists.With the development of laser technology, its clinical application in the field of acne scar is increasing year by year.However, there is a lack of bibliometric analysis on the laser treatment of acne scars. The objective of this study is to utilize bibliometrics to gain a comprehensive understanding of the development trends and research hotspots in the field of laser treatment for acne scars.

Method : With "acne scar*" and "laser" as the themes, the publications about laser treatment of acne scar from 2014 to 2023 were searched in the WoSCC database. The literatures were visualized by VOSviewers, CiteSpace and R software packages, and the maps of countries, research institutions, authors, journals, references and keywords were drawn

Results: A total of 430 articles from 46 countries were included in the analysis, with the United States and China taking the lead. The main research institutions are Mahiron University in Thailand, Cairo University in Egypt and Chulalongkorn University in Thailand. "Journal of Cosmetic Dermatology" became the journal with the largest number of articles in this field, and "Dermatologic Surgery" became the most cited publication.A total of 1695 authors contributed to these publications, with Manuskiatti Woraphong being the most prolific contributors. Notably, Alster Tina received the highest co-citations. Research on the effectiveness of lasers in treating acne scars is a significant area of focus in this field. Among the various types of lasers, CO2 lasers are the most commonly utilized.Emerging research hotspots include various types of lasers, as well as microneedle radiofrequency and combination therapy

Conclusion: Treatment effectiveness is the focus of research in this field. Combination therapies such as laser, microneedle combined with platelet-rich plasma, hyaluronic acid and new picosecond lasers have gradually become a hot topic in this research field and trend

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Recent Progress on Phototherapy and Fluorescent Imaging Probes

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The development of fluorescent probes for various analytes has been actively pursued by chemists. Since their inception, these efforts have led to many new sensors that have found wide applications in the fields of chemistry, biology, environmental science, and physiology. Recently, a near-infrared two-photon fluorescent probe was developed to not only specially image carboxylesterase (CE) activity *in vivo* and *in situ* but also target orthotopic liver tumor after systemic administration.[1]

On the other hand, photodynamic therapy (PDT) and photothermal therapy (PTT) have attracted considerable interest as a noninvasive treatment method.[2] We devised a novel molecular design approach to create heavy-atom-free photosensitizers for thionapthalimides.[3] The *in vivo* specific binding between albumin and PcS, arising from the disassembly of injected NanoPcS, was also confirmed using an inducible transgenic mouse system.[4] We recently reported a viscosity-sensitive, endoplasmic reticulum (ER)-targeting fluorescent probe, ER-ZS, which can monitor ER stress-induced viscosity changes in real time. ER-ZS is also an excellent anti-hypoxia type I photosensitizer that activates tumor cell pyroptosis by damaging the ER pathway.[5]

Photodynamic antibacterial therapy is regarded as an innovative and promising antibacterial approach due to its minor side effects and lack of drug resistance.[6] Recently, we suggested that reactive differences may pave a general way to design selective photodynamic agents for ablating Gram-positive bacteria-infected diseases.[7]

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New treatment option for rectal cancer: X-ray activated photodynamic therapy

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Rectal cancer accounts for approximately 30% of colorectal cancers which is the 2nd most deadly cancer in Australia [1]. A significant proportion of Stage II-III rectal cancer patients undergo chemo-radiotherapy to downsize the primary tumour and lymph node metastasis before surgery [2]. Unfortunately, the side effect of this standard of care treatment is progressive late morbidity due to high doses in long-course radiotherapy and toxicity of chemodrugs used in chemo-radiotherapy [3]. To improve the quality of life of rectal cancer patients, it is important to find a new and safe treatment method. Here we brought a new treatment method, X-ray triggerable photodynamic therapy (X-PDT), by combining existing clinical techniques used in cancer treatment and delivering via bespoke nanocarriers [4]. In this strategy verteporfin (VP), a clinically approved photosensitizer, was directly activated by X-ray via a nanoplatform. The activated VP generates highly toxic reactive oxygen species, killing the cancer cells. With X-PDT, the tumour growth suppression was observed in an orthotopic mouse model bearing rectal cancer. Such tumour control is consistent with decreased cell viability, increased necrotic tumour tissue and reduced Ki-67 protein expression observed in the mouse group treated with X-PDT, compared with other treatment conditions. Our study establishes an effective strategy to treat rectal cancer in a more clinically relevant model, which offers prospects for clinical translation of this technology for deep seated cancers.

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A new nanoformulation of verteporfin for photodynamic therapy of glioblastoma

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Publish consent withheld

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Dynamics and mechanism of UVR8 dimer dissociation

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

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Role of BBX proteins in UV-B signaling

Sourav Datta[1]

1. Invited , Speaker

Abstract Content TBC

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Photooxidation-Induced Weathering and Fragmentation of Thermoplastics under Simulated Sunlight Exposure

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Microplastics are widespread in the environment, formed through the gradual weathering and fragmentation of larger plastics into nanoplastics (NPs) and microplastics (MPs). However, the weathering process and fragmentation rate remain poorly understood. To address this, we quantitatively determined NPs (60-800 nm) and MPs (0.8-500 μm) produced from thermoplastics by accelerated sunlight simulated photodegradation in air and water environment. The initiation of fragmentation of low-density polyethylene (LDPE), polypropylene (PP) and polystyrene (PS) were determined over 240 days in air, and the fragmentation rates of virgin and additive-containing PP before and after mechanical abrasion in water were compared. The acceleration factor of the solar simulator, compared to conditions in South Korea, was calculated as 5.5.

The initiation of fragmentation by photooxidation was in order of PS (< 1 year), PP (< 2 years) and LDPE (> 3 years) in South Korea by sunlight exposure. Despite PS exhibiting the fastest initiation of fragmentation, photodegradation rates, total particle abundance, and increasing ratio (exposure/non-exposure) were comparable or lower than those of PP. The fragmentation rate of PP and additive-containing PP appeared similar after 176 days of simulated sunlight exposure followed by mechanical abrasion (equivalent to 2.7 years of outdoor exposure in South Korea). Additionally, mechanical abrasion from vortexing played a significant role in the production of MPs, whereas it had a smaller impact on the generation of NPs.

These results suggest that weathering and fragmentation by photooxidation of PP are more rapid and effective in air than in water. The initiation of fragmentation was faster in air (approx. 1.8 years) than in water (> 2.7 years). The fragmentation rate of thermoplastics determined in this study provide valuable insights for estimating secondary microplastic production through weathering, informing decision-making regarding timely plastic litter removal from the environment.

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Harnessing, protecting from, and evaluating effects of UV radiation: Case studies of cellulose nanocrystals, nanodiamond-laden gels and biodegradable polymers

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UV radiation produces a wide range of effects with it being more readily known for its deleterious impact; however we can also utilize it to our advantage. In this presentation, we discuss three scenarios for UV radiation. In the first case, *we discuss cases where UV radiation can be exploited to develop functional polymer gels and coatings*. We discuss how UV curable coatings can be developed by understanding the effects of UV radiation on the rheology of polymers. In particular, we will explore the gelation of these systems as a function of UV radiation. Two systems of interest would be alginate- and cellulose nanocrystals (CNCs)-based materials. The sol-gel transition, evidence of dark curing, formation of percolating networks and gelation mechanism will be elucidated. In the second case, we *discuss the use of nanodiamonds to protect us from UV radiation.* NDs are carbon-based multifunctional nanomaterials that contain a sp3 hybridized core, a graphitic outer shell and multiple surface functional groups that can be readily tuned. The high refractive index of NDs promotes light scattering while the functional groups and graphitic shell absorb UV photons, allowing broad-spectrum UV filtering. In this case, we show how carboxylated nanodiamonds (cNDs) modulate the rheology and UV attenuation properties of polyacrylic acid (PAA) microgels through pH and concentration modulation. The unique aspects of our work lie in a) tunability of microgel rheology and lubrication, through controlled modulation of pH and cND concentration, b) an understanding of the underlying interaction mechanism of pH-tunable microstructural reinforcement, c) broad-spectrum, photostable UV protection by cNDs comparable to TiO2 , d) preservation of gel microstructure after prolonged UV irradiation, all of which leading to e) a safer formulation (e.g., for sunscreen and topical lotions) using multifunctional nanodiamonds. In the final case, *we discuss how UV radiation affect polylactic acid (PLA) degradation*. Our choice for PLA stems from its appeal to replace more traditional polymers. Approaches to measure PLA degradation under aerobic and anaerobic conditions following UV radiation, and possible mechanisms involved will be discussed.

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Flicker and other effects of Temporal Light Modulation (TLM)

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Temporal variation in the light output of light sources and lighting systems (temporal light modulation, TLM) is sometimes a feature (e.g. signal flashes) but more often is an undesirable side-effect of product or system design and/or operation. TLM explains the association of magnetically-ballasted fluorescent lighting systems with headache and eyestrain complaints. These complaints largely disappeared following the adoption of electronic ballasts, but the introduction of light-emitting diode (LED) lamps and lighting systems has renewed researcher, manufacturer, and regulator attention to TLM.

This presentation will provide a brief overview of some of the visual, behavioural, and neurological problems that TLM can cause, identifying some of the most urgent research questions and describing why TLM is a very difficult lighting parameter to measure and to investigate.

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How is the RPE melanin modified during the life-long exposure to sunlight?

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The retinal pigment epithelium (RPE) cells protect retina from oxidative stress. The deterioraton of its protective function may lead to the etiology of age-related macular degeneration. It is known that the amounts of RPE melanosomes decline with age. However, the molecular mechanisim of this phenomenon and the structural changes of the modified melanin remain little known. Melanocytes produce two types of pigment, eumelanin and pheomelanin. Eumelanin consists of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA), while pheomelanin consists of benzothiazine and benzothiazole units. Melanins can be analyzed through specific degradation products by HPLC. Alkaline hydrogen peroxide oxidation (AHPO) of eumelanin gives pyrrole-2,3,5-tricarboxylic acid (PTCA) and thiazole-2,4,5-tricarboxylic acid (TTCA) as specific degradation products of DHICA moiety of eumelanin and benzothiazole moiety of pheomelanin, respectively. AHPO of synthetic and natural melanins show that the photoaging of eumelanin gives rise to pyrrole-2,3,4,5-tetracarboxylic acid (PTeCA, produced by cross-linking), leading to the increase in the ratio of PTeCA/PTCA. Benzothiazine pheomelanin can be analyzed by reductive hydrolysis, as 4-amino-3-hydroxyphenylalanine (4-AHP) and 3-amino-4-hydroxyphenylalanine (3-AHP). In this study, we compared changes in various melanin markers and their ratios in human melanocytes exposed to UVA, in isolated bovine RPE melanosomes exposed to strong blue light and in human RPE cells from donors of various ages. The results indicate that the PTeCA/PTCA ratio is a sensitive marker for the photo-oxidation of eumelanin and that both eumelanin and pheomelanin in human RPE cells undergo extensive structure modifications/degradations due to the life-long exposure to blue light. The decreased amounts of eumelanin could lead to deterioration of its ability to protect RPE cells against oxidative stress. Further, because of the generation of a more complex, sterically crowded 3D structure, the degraded eumelanin might have a lowered potential to sequester toxic iron and to scavenge reactive oxygen species.

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Cyclic AMP-regulatory element-binding protein: A novel early marker that could predict the efficacy of sun protective agents in reducing skin carcinogenesis.

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Australia and New Zealand have the highest skin cancer rates worldwide, despite ongoing sun safety campaigns. It is therefore necessary to enhance current photoprotective measures. We have demonstrated that active vitamin D compound, 1,25-dihydroxyvtamin D_3 (1,25D), and structurally related compounds including 20-hydroxyvitamin D (20D), 1,25-dihydroxylumisterol (JN), QW-1624F_2-2 (QW) and tetrahydrocurcumin (THC) are novel photoprotective agents. It has generally been accepted that agents capable of protecting against acute markers of ultraviolet radiation (UVR)-induced damage such as DNA damage and immunosuppression, would provide protection against chronic UVR damage in a 40-week murine photocarcinogenesis protocol. Several photoprotective agents including 20D and QW, however, have proven otherwise. Therefore, markers of acute UVR-induced damage are not reliable predictors to predict the ability of a photoprotective agent to protect against photocarcinogenesis. Phosphorylated Cyclic AMP-regulatory element-binding protein (pCREB) is a transcription factor that is overexpressed in skin cancer. We have shown that UVR increases pCREB levels in melanocytes and keratinocytes. Considering this, pCREB may be a potential predictor of the ability of a photoprotective agent to protect against photocarcinogenesis. Our studies in primary human dermal fibroblasts showed that 1,25D treatment immediately after UVR exposure significantly reduced pCREB levels ($p < 0.05$). This was supported by *in vivo* studies showing significant reductions in UVR-induced pCREB levels in Skh:hr1 mouse skin following topical application of 1,25D or related vitamin D compounds JN and THC ($p < 0.01$). Conversely, compound 20D, which did not prevent photocarcinogenesis, did not prevent UVR-induced increase in pCREB (p = ns). Preliminary studies in *ex vivo* human skin have demonstrated a similar trend with 1,25D treatment causing reductions in pCREB levels following UVR exposure. These results demonstrate that pCREB may potentially be a predictor of photocarcinogenesis and could streamline the process of identifying suitable photoprotective agents for a 40-week photocarcinogenesis model.

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Efficacy of oral nicotinamide monotherapy versus combinational treatments in the prevention of ultraviolet radiation-induced skin cancer

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Keratinocyte carcinomas (KC) are cancers of the skin predominantly caused by ultraviolet radiation (UVR). Oral nicotinamide (Vitamin B_3; NAM) is reported to reduce KC and pre-malignant lesions in healthy volunteers. Here, we report the efficacy of three monotherapies (NAM, phloroglucinol [PG], and metformin [Met]) and two combinational treatments (NAM-PG and NAM-Met) in preventing UVR-induced skin cancer. Female hairless C3.Cg-*Hr*^{hr}/TifBom Tac mice were exposed to UVR of 3.5 standard erythema doses three times a week to induce skin cancer development. In three subsequent experiments, mice ($n = 25$ per group) were treated concurrently to UVR with i) 600 mg/kg NAM or 100 mg/kg PG, ii) 600 mg/kg NAM or 300 mg/kg Met, iii) 600 mg/kg NAM, 75 mg/kg PG + 400 mg/kg NAM, or 200 mg/kg Met + 400 mg/kg NAM. In all three experiments, a UVR control group was also included. Our data showed significant protection provided by PG and NAM monotherapies compared to the UVR control group ($p \leq 0.036$). Met monotherapy did not reduce tumour development ($p > 0.05$). In the combination study, all three treatments

protected against tumour development ($p \leq 0.015$). NAM-Met was less effective compared to NAM-monotherapy ($p \leq 0.01$), indicating inferior photoprotection. In contrast, NAM-PG reduced pyrimidine-pyrimidone (6-4) photoproducts measured by immunohistochemistry in comparison to both the UVR control group (40%; $p \leq 0.0021$) and NAM-monotherapy (37%; $p \leq 0.0082$). Our data indicate that the combination of two treatments (NAM and PG) provides effective pre-clinical photoprotection even at a reduced dose. The reduction in DNA damage could suggest that NAM and PG may synergise to mediate an improved protective mechanism better suited for keratinocyte carcinoma prevention.

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Mueller matrix-based label-free measurement of structures of skin

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Anisotropic structures, such as fibers, are highly important components of skin, providing functions such as strength, elasticity, and protection. They are sensitive to polarized light, and their polarization response characteristics can be expressed by Mueller matrix which could be measured by the changes of the polarization states of light. Mueller-matrix optical coherence tomography and Mueller-matrix polarimetry are two typical label-free techniques that can acquire cross-sectional Mueller matrices and surface Mueller matrices of tissues. By using these two methods, the polarization information of normal skin, malignant melanoma, malignant lentigo, etc. can be analyzed and compared with their pathological results. The sensitivities of different polarization parameters of the skin can be studied in order to find some specific ones that can better assist clinical diagnosis.

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N6-Methyladenosine Modification in UVB-induced Cellular Senescence of Skin Photoaging

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Publish consent withheld

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Phototherapy of cutaneous T-cell lymphomas

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Mycosis fungoides (MF), the most common variant among cutaneous T-cell lymphomas (CTCL), is characterized in its early stages by clonal proliferation of malignant T-cells in the skin, which manifest with erythematous patches and plaques. In some patients, progression occurs during the chronic course with cutaneous tumors and involvement of extracutaneous organs. Skin-directed therapies (SDT) are primarily used in the early stages of the disease. Among SDT, phototherapy with ultraviolet A radiation in combination with 8-methoxypsoralen (PUVA) and ultraviolet B radiation (UVB) have a long tradition in the treatment of MF and are highly effective in achieving remission. Sézary syndrome (SS) is a rare and more aggressive CTCL variant with generalized skin involvement. Patients with SS and with erythroderma from MF can benefit from treatment with extracorporeal photochemotherapy (ECP) where peripheral blood is exposed to PUVA. Another photoresponsive CTCL variant is lymphomatoid papulosis (LP), a CD30+ lymphoproliferative disease characterised by chronically recurring papules. LP responds favourably to PUVA but low dose methotrexate might be preferred for long term disease control. Phototherapy can be safely combined with systemic agents, especially interferon-alpha and retinoids.Recently, updated treatment guidelines have been published that include evidence-based algorithms for the stage-oriented treatment of MF. PUVA and narrow-band UVB (NB-UVB) are recommended as initial treatment for early stages, while combination treatments are reserved for refractory and more advanced cases; ECP is mentioned among the standard treatments for MF erythroderma and SS. Uncertainties exist regarding optimized treatment dose and schedules, the use of phototherapy for maintenance, and the role of newer phototherapeutic modalities (e.g. ultraviolet A1 radiation, excimer sources, photodynamic therapy) in the treatment of CTCL.

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Photodynamic Therapy of Non-Oncologic Skin Conditions

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Over the past years, photodynamic therapy has been widely used in dermatology mainly for oncologic indications. However, first reports of its efficacy in non-oncologic skin conditions were reported already in the past century, and even the work of the first Nobel prize winner, Niels Ryberg Finsen (phototherapy of lupus vulgaris (skin tuberculosis)) has been identified to rely on the photodynamic principle.

Unfortunately, so far there is no regulatory acceptance of PDT for non-oncologic indications in dermatology. In recent guidelines, and based on field-directed treatments for actinic keratoses, aesthetic aspects with improvement of age-related skin changes

reached an excellent grading according to quality of studies and level of recommendation. Other approaches with larger experiences are in the field of microorganism-associated skin diseases like cutaneous leishmaniasis and fungal infections. Current treatment protocols also study the effects of PDT in acne and rosacea. One possible drawback is the presence of relatively cheap alternatives and the need of repetitive treatment approaches which is cumbersome and associated with multiple side-effects.

Perhaps there will be an enlivenment of PDT also in this field based on the new developments in the treatment itself: Daylight-PDT, or artificial daylight-PDT enables the treatment of large areas without painful sensations which is one important prerequisite for patient's acceptance.

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Human exposure to Far-UVC: balancing risk and benefit

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Terrestrial ultraviolet radiation (UVR) exposure has profound effects on human skin, notably sunburning, photoaging, immunosuppression and photocarcinogenesis. Less attention has been paid to the effects of UVC as we are not naturally exposed to this due to ozone absorption. However, germicidal UVC (254 nm) potently inactivates pathogens and is employed for disinfection purposes but is harmful to human skin and eyes. The development of artificial sources of filtered UVC (Far-UVC; 222 nm) through Krypton Chloride excimer light technology, accelerated during the pandemic given the virucidal effects of Far-UVC and its apparent safety, and commercial roll out of these devices has meant that large scale artificial environmental human Far-UVC exposure is on the horizon in public places. This roll out has exceeded our understanding of the importance of human safety of Far-UVC, and whilst potential major benefits of disinfection using Far-UVC are feasible, we need to balance this with the potential risk to human health, and this is a critical public health issue.

Far-UVC has very limited skin penetration effects, and initial human skin studies indicating safety have been promising. We undertook a two-stage study of the visual and histological effects of Far-UVC in healthy volunteers using single and repetitive exposures to virucidal Krypton Chloride Far-UVC-emitting excimer lamps (PIVUC), using UVB as positive control in a within-subject controlled trial, with visual, reflectance and histological end points, focussing on DNA damage. No significant visual or pathological changes, including cyclobutane pyrimidine dimers and Ki67 staining, were seen (n=20). We have subsequently also seen no visual changes in patients with photosensitivity diseases.

Whilst we need to be cautious in terms of extrapolating these data to larger scale human exposure, this evidence is encouraging with respect to the human safety of Far-UVC, although we must keep an open mind.

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Natural Nanoparticle-Photosensitizer complexes: From design to photodynamic therapy (PDT) application.

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In recent years, in Limoges lab, we have been developing the design and synthesis of nanoparticles of natural origin for the delivery of photosensitizers (PS) (porphyrins, chlorines, curcumin, etc). These nanoparticles are made from polysaccharides in order to have a hydrophilic character to prevent opsonization by macrophages. In addition, the presence of osidic units on these polysaccharides means that alcohol functions are available and can be functionalized. These nanoparticles, based on dextran, cellulose or hemicellulose, were characterized by TEM, DLS and zeta potential. Recently, cellulose nanoparticles (Cellulose NanoCrystals or CNCs) in needle form have shown very good results for uptake of photosensitizer in different in vitro cancer cell lines and could be a very nice nanoplatform for drug delivery. Preliminary investigations have been realized *in vivo* with two types of PS/nanoparticles.

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Porphysome Nanotechnology: Beyond Lab, Beyond Light and Beyond Cancer

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Porphysomes are liposome-like nanoparticles self-assembled from a single porphyrin-lipid building block, which enables their inherent multifunction of photothermal, photoacoustic, photodynamic, fluorescence, MRI, PET, SPECT, and radionucleotide therapy. Since its discovery, we have demonstrated porphysome's high tumor selectivity and multimodal theranostic utilities in diverse tumor models and animal species. We have completed GMP manufacturing, GLP safety studies and clinical trial protocols for its first-in-human use, aka 'beyond lab'. We have also developed a suite of next generation porphysomes that greatly broadened its theranostic applications from light to sound to radiation. These allow us to pursue new directions of 'beyond light' and 'beyond cancer'.

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Plants to humans: Arabidopsis for translational research

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Repeat sequences are present in plants to human to microbes, and unnatural repeat expansions are associated with several linked to several human genetic disorders. Expanded repeats carry peculiar features; repeats in either the noncoding/coding region often affect gene expression and protein function. However, the underlying mechanism of the repeat expansion-induced gene expression remains unclear. One of the problems is the lack of an appropriate model to study since most of the expansions are known primarily in human genetic diseases. Arabidopsis turns out to be the first non-human model that shows a trinucleotide repeat expansion-associated phenotypic variation. The Arabidopsis wild strain harbors a triplet repeat expansion associated with a growth defect associated with the downregulation of gene expression. Over the years, we have developed Arabidopsis as a natural method to uncover the molecular mechanisms associated with repeat expansion-induced downregulation of gene expression. Recently, we discovered that repeat expansion leads to accumulation of 24nt siRNAs, which via RNA-directed DNA methylation (RdDM) pathway induces epigenetic gene silencing at repeat expanded genetic locus harbouring the expansion. However, several questions remain unanswered, including how cellular variability contributes to gene regulation. To address this gap, we performed a genetic suppressor screens and identified several genetic suppressors, which confirm the role of RdDM pathway and reveal novel components that are essential for repeat expansion-induced epigenetic gene silencing. Here, I will describe the novel genes and pathways we have uncovered that regulate repeat expansion-associated gene regulation. Our findings provide important insights into the molecular mechanisms underlying the association between trinucleotide repeat expansion and pathogenesis in Arabidopsis. Overall, our study has significant implications for understanding the underlying mechanisms of repeat-induced gene regulation and the pathogenesis non only in the plants, would aid in understanding the human genetic disorders associated with trinucleotide repeat expansions.

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Functional characterization of tomato COP1, COP1h and SPA3 in UVR8-mediated UV-B signaling

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Tomato (Solanum lycopersicum) is an important horticultural crop worldwide. UV-B signaling is extensively investigated in the model plant Arabidopsis thaliana, however, much less is explored in crops. We investigate UV-B signaling in tomato in our laboratory. We generated a series of tomato mutants including *uvr8*, *cop1*, *cop1-homolog*, *spa3* and *hy5* with CRISPR/CAS9 approach. With these major mutant lines, we compared UV-B signaling in Arabidopsis and tomato. We found both commonanity and specificity of UV-B signaling in these two plant species. I will present our recent data of UV-B signaling in tomato plant development and metabolism.

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The design of nanomaterials for degradation of plastics and toxic compounds by UV-radiation

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The effects of UV-radiation on materials can be complicated. On one side, the UV-radiation, especially the UV-B radiation with short wavelengths and high energy, will cause the degradation of materials, which draws the attention to design materials that can resist the degradation providing appropriate life time for application. On the other side, the UV-radiation can be applied as natural energy to assist the degradation of the toxic compounds and waste materials. In this talk, we will talk about how we design nanomaterials for the efficient degradation of toxic compounds and plastic in consideration the role of UV-radiation.

Tetracycline hydrochloride (TCH) with a stable benzene ring skeleton structure and high hydrophilicity, rendering its resistant to degradation in aqueous environments. In the first work, we designed and synthesized a composite photocatalyst nanomaterial via the hydrothermal method by integrating the carbon quantum dots (CQDs) derived from carbon aerogel (CA) into BiOCl. The resulting composite photocatalyst introduced an abundance of oxygen vacancies (OVs), leading to enhanced catalytic degradation performance. Experimental results demonstrate that CA/BiOCl nanocomposite exhibits a pronounced photocatalytic effect, showcasing a degradation rate of tetracycline hydrochloride (TCH) under a full spectrum irradiation, achieving a degradation rate of ~100%.

In another work, we designed a double-side catalyst based on titanium oxide, which plays key roles in both polymerization and depolymerization of polyethylene terephthalate (PET, accounts for more than 80% of chemical fibers). The effects of UV-radiation including the intensity and spectral composition on degradation of titanium catalyst contained PET plastics will be introduced. The perspective and challenges in using such nanomaterials as additives for the preparation of functional fiber materials will also be discussed.

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Photodegradation of Plastics and Wood-Plastic Composites under Desert Natural Weathering Conditions

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The durability of two sets of Wood-Plastic Composites (WPCs), one based on polypropylene (PP) another on high-density polyethylene (HDPE) was investigated. Injection molded samples of the WPCs with different loadings of wood fiber ranging from

0 to 36 wt. percent of wood were subjected to laboratory accelerated weathering as well as natural weathering under harsh desert exposure conditions in Dhahran, Saudi Arabia. The integrity of samples weathered to different extents were tested using standard tensile test and surface hardness test to investigate the dependence of these properties on the duration of weathering exposure. The average tensile strength of all composites as well the respective controls decreased monotonously with weathering exposures. Their tensile moduli increased with weathering indicating extensive crosslinking and possible increased crystallization during the weathering exposure. Changes in hardness with exposure also indicated a similar decrease under both exposure regimens. The changes obtained compare well with those reported for WPCs using a different wood fiber. An interesting correlation between hardness and tensile properties was obtained for PP-based composites.

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International Union of Photobiology (IUPB), the Molecular and Experimental Pathology Society of Australasia (MEPSA) World Congress 2024

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Toxic synergistic toxicity between blue light and atmospheric pollutants for the retina

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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 retina. 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. HEV-light absorption by nanomolar concentrations of IcdP present in retinal cells promotes degenerative changes comparable to the ones observed in 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 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 Nrf2-controled 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.

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Melanopic equivalent daylight illuminance regulated by smart shading in office buildings

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Publish consent withheld

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The right light at the right time in the right place: Optimising lighting to promote well-being

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Light is any electromagnetic radiation that can create a visual sensation by directly stimulating the retinal photoreceptors of the visual system. In addition to enabling vision, these photoreceptors also connect to diverse brain locations through which light triggers biological effects that powerfully regulate human health, performance and well-being.

Exposure to light can improve alertness, influence thermoregulation, and alleviate seasonal and non-seasonal depression. Light is also the main synchronizer of the human biological clock. However, depending on the timing and intensity, light exposure can shift the phase of the circadian rhythm and can regulate the timing and quality of sleep. Light in the evening and at night can disrupt sleep and can cause acute suppression of the nocturnal release of the hormone melatonin. The term “integrative lighting” is used as the official term for lighting that is specifically intended to integrate visual and non-visual effects, producing physiological and psychological effects on humans that are reflected in scientific evidence.

This presentation will outline how to characterize light with respect to its integrative effects, how to identify the right light at the right time in the right place as a means of maintaining and promoting health, performance and well-being, and how to deliver that light in a practical and energy-efficient manner.

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Photobiological mechanisms in the development and treatment of multiple sclerosis

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Inflammatory skin conditions such as psoriasis have been successfully treated for decades with narrowband UVB radiation (NB-UVB). The prevalence of multiple sclerosis (MS) has shown latitude gradients with more disease at higher latitudes where there is less ambient UV radiation. In the PhoCIS trial (Phototherapy for Clinically Isolated Syndrome), participants with CIS, the earliest detectable form of multiple sclerosis (MS), were randomised into a treatment arm with exposure to suberythemal NB-UVB (311-312 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 used a biobank of frozen cells and sera from the participants to investigate the mechanisms by which NB-UVB may alter immune cell networks and alter MS progression. Alternatively, biomarkers associated with exposure of skin to NB-UVB may be uncovered. Both the proteome and the metabolome have been analysed in sera taken longitudinally from the participants receiving NB-UVB, compared with the control non-irradiated CIS participants. This has been complemented by studies of biomarkers in serum that have been clinically validated to give predictions of new lesions indicative of MS progression. These biomarkers have confirmed the clinical and radiological benefits of exposure to NB-UVB.

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

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Lighting the way: Cluster-triggered emission materials as an upstart for the development of biophotonic applications

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The ongoing search for innovative and environmentally friendly luminescent materials with tunable physical and chemical properties at the nanoscale is in high demand. Their unique properties, such as low toxicity, excellent biocompatibility, and tunable cluster-triggered emission luminescence (CLgens) in response to external stimuli, make them ideal probes for biophotonic applications.[1] However, the relationship between the chemical structure of CLgens and their unexpected optical properties is still the missing link to enhance their applications. This study aimed to relate the chemical structure of the newly synthesised CLgens from biomass-derived monomers such as carvone. The spectroscopic studies at steady-state and time-resolved levels help unravel the structure-properties and how this is affected by an external physical stimulus such as temperature and, later, pH. Based on these promising results, and as a proof of concept, the thermal sensitivity was successfully tested at the microscale level to visualise how temperature rises in photothermal methods.[2] So, if the luminescent properties of CLgens do not lack properties previously associated only with traditional chromophores, can they be extrapolated to other types of excited state applications? The real question is: could these excited states transfer energy to oxygen and form reactive oxygen species (ROS)? The preliminary results on the carvone polymers suggest that this is possible and that the long-excited lifetimes could efficiently transfer their excess energy to molecular oxygen to produce ROS such as $^1O_2$. Still, more importantly, they have excellent photoantimicrobial capabilities against Staphylococcus aureus (*S. aureus*). Therefore, the aggregated results highlight the considerable potential of CLgens as light-emitting materials and mark a crucial turning point in creating a new wave of photosensitizers.

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Using photochemistry to help solve problems in photomedicine and photobiology: Mechanistic details

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My talk will focus on fundamental and applied topics in the photosciences. First, a photoconversion of heptamethine cyanine to trimethine cyanine will be described that involves singlet oxygen and subsequent 2-carbon or 4-carbon truncations. The latter truncation appears to proceed through an allene hydroperoxide intermediate and then a downstream retro-Diels-Alder reaction for a potentially useful photobluing optical tool ($\Delta\lambda_{em}$ of ~200 nm). Mechanistic details on this somewhat unusual cyanine phototruncation will be provided. Secondly, the development of a hand-held fiber device will be described which delivers singlet oxygen but not sensitizer by a superhydrophobic-tip. Red light emitted by the fiber tip is transmitted through the backside of a polydimethylsiloxane strip before irradiating a verteporfin coating facing a biofilm-covered tooth or gingival surface. The device shows promising results based on a Wistar rat model of periodontitis. Results will be highlighted from our collaborations with the groups of Martin J. Schnermann (National Cancer Institute), Alan Lyons (College of Staten Island), and Tayyaba Hasan (Harvard Medical School).

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Enhanced singlet oxygen generation for Hemoporfin-mediated photodynamic therapy

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Photodynamic therapy (PDT) involves the administration of a tumor-localizing photosensitizer (PS) followed by light irradiation with a specific wavelength in the presence of molecular oxygen, leading to the generation of cytotoxic singlet oxygen causing the irreversible destruction of malignant and nonmalignant diseases. Hemoporfin is a clinical approval PS widely used for port wine stain and skin diseases in China. In order to enhance singlet oxygen generation for Hemoporfin-mediated PDT, liposomes were utilized as the carrier for Hemoporfin to prolong the blood circulation time and enhance the accumulation *in vivo*. In addition, the L-buthionine sulfoximine (BSO) and catalase (CAT) were loaded within the hydrophilic cores of liposomes to obtain the BSO/CAT@Liposome-Hemoporfin nanoparticles (BCHL-NPs). The enhanced singlet oxygen generation was sucessfully achieved both in vitro and in vivo, which could be mainly attributed to the increase of Hemoporfin and oxygen concentration in the tumor and the depletion of intracellular glutathione. As a result, the enhanced singlet oxygen generation after BCHL-NPs mediated PDT could significantly suppress the tumor growth without additional side effects as comapred to Hemoporfin.

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Photochemical strategies to overcome hypoxia in photodynamic therapy

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Oxygen is widely acknowledged to play a critical role in photodynamic treatments, which often fail due to hypoxia. Researchers have made significant efforts to ensure an adequate oxygen supply during the illumination phase of PDT. Interestingly, some photosensitizers have been found to remain effective even under hypoxic conditions. The objective of this study was to understand the molecular mechanisms behind this behavior and identify design principles for photosensitizers to overcome hypoxia challenges.

The photophysics and photochemistry of hypoxia-active compounds have been studied by steady-state and time-resolved spectroscopic methods. The anticancer and antibacterial activity has been studied by standard cell phototoxicity assays. Mechanistic insight has been gained by the use of confocal microscopy and reactive oxygen species fluorescence probes.

The optical, excited-state, and redox properties of hypoxia-active photosensitisers have been determined, as well as their anticancer and antimicrobial activity, both under normoxia and hypoxia conditions. Distribution of ROS species has been determined in each case.

The main determinants of photodynamic activity under hypoxia are a strong photoredox capacity and the formation of long-lived intermediates upon photoexcitation.

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PhotoDynamic Therapy Dosimetry: Where from? Where to?

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Accurate dosimetry has been a theme throughout the development of PDT to achieve optimal, repeatable treatment delivery. This can be relatively straightforward, such as in surface illumination using topical photosensitizer, where the administered light and drug doses can be easily standardized. However, for “volumetric” treatments of, for example, solid tumors or for large-area intracavitary treatments, the complex geometries, the need for multiple light sources and the heterogeneity of tissue optical properties, photosensitizer uptake and oxygenation, require more complete and rigorous pre-treatment planning as well as real-time *in situ* measurements to correlate with outcomes.

Multiple dosimetry approaches have been developed, including a) calculating/measuring the light fluence distribution, photosensitizer uptake and/or oxygenation and combining these in an ”effective-dose” model, b) measuring/imaging photosensitizer photobleaching as surrogate metric, and c) directly or indirectly detecting the singlet oxygen generation. The status, advantages and limitations of each approach are considered, including clinical practicality and utility.

The underlying assumption has been that there is a direct correlation between the effective PDT dose and the resulting outcome and evidence supports this in many cases. However, in some cases the effective dose has been a poor indicator of outcome. This response variance for a given “dose” has limited wide clinical adoption of PDT, particularly in oncology.

One factor that has likely contributed to the response variance is secondary immune regulation induced by the inflammatory processes resulting from PDT. There is evidence in preclinical tumor models that this can be the dominant, and highly favorable, mechanism, both for the primary tumor outcome and to impact tumor progression and metastases. However, this “PhotoChemical Immune Stimulation (PCIS)” essentially decouples outcome from the PDT biophysical dose. Hence, PCIS dosimetry presents major new dosimetric challenge that will need to incorporate not only the biophysical parameters but also the photobiological and immunological factors.

Brian C Wilson, Princess Margaret Cancer Centre-University Health Network/University of Toronto, CA

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Optimal positioning of cylindrical light distributors used for interstitial PDT

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Interstitial photodynamic therapy (PDT) and photoimmunotherapy (PIT) are frequently based on the use of cylindrical light distributors (CLDs). PDTs and PITs of large tumors require treatment planning to maximize the therapeutic dose spatial distribution while minimizing the number of CLDs. This is, in particular, the case when CLDs are inserted in parallel to treat head and neck squamous cell cancers. In this presentation, we will describe how to position CLDs to maximize the necrosed volume of such cancers for different CLDs insertion geometries. In addition, we will describe the influence of different tissue optical parameters on these positions, in particular when head and neck squamous cell cancers are treated by interstitial PIT with cetuximab–IR700. The descriptions of the light propagation around CLDs were performed using Monte-Carlo simulations with tumor optical properties derived from the literature, when CLDs were inserted perpendicularly to the air-tissue interface. These simulations enabled to determine the tumor volume receiving light doses larger than a therapeutic threshold. An optimization algorithm was then developed to calculate and maximize the necrosed tumor volumes.

Our results indicate that the values of the absorption and reduced scattering coefficients have the most significant influence on the optimal CLD positions. In contrast, the tissue anisotropy factor, the CLD insertion patterns and length, as well as the angular dependence of their radiances have minimal influences. Finally, at first approximation, the variations of the optimal CLD-CLD distances due to changes of their length, optical properties of the tissue, and choice of geometry, are decoupled.

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Interstitial PDT treatment planning: Managing dosimetry with heterogeneities and uncertainties.

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At present, the intended goal of PDT dosimetry is to curtail or modify the optical energy delivery throughout the clinical target volume following a predetermined photosensitizer administration and empirically determined light source spacing under consideration of the anatomical limitations. The underlying assumption is that the photosensitizer’s specific uptake ratio in the target over the surrounding normal tissues is large enough to compensate for intra and interpatient variability in the dose due to differences in the photosensitizer accumulation and local pO_2.

Treatment failure is often attributed to insufficient local or global photosensitizer and/or oxygen concentration in the clinical target volume.

Monitoring the photosensitizer fluorescence during interstitial PDT may undersample the spatial distribution of the photosensitizer concentration, and the current empirical placement of interstitial light sources can not mitigate this spatial dose variability, particularly at the edge of the target volume.

Knowledge of the spatial photosensitizer and oxygen availability is desirable for providing patient-centred PDT light treatment planning. However, what spatial resolution is required to derive different treatment plans? How can the spatial oxygen and photosensitizer distribution be predicted prior to the placement of the light emitters?

Ongoing *in silico* studies aim to determine the impact of varying spatial resolution in the photosensitizer and oxygen concentration quantification on PDT treatment planning solutions. The variability of the photosensitizer concentration is modelled based on in vivo fluorescence imaging studies of intracranial and subcutaneous tumour models. Comparison with fMRI studies imaging blood flow and blood volume indicate that the mean transit time of blood is a good indicator for the local photosensitizer accumulation.

Hence, the spatial quantification of blood flow and volume as biomarkers for photosensitizer and oxygen availability can be used to for the local PDT dose description, leading to improved tumour eradication.

What are the remaining uncertainties? Independent of how many PDT efficacy-determining parameters the ‘dose’ definition is based on, the influence of other stressors and the biological microenvironment on the minimum dose to cause cell death remains poorly studied.

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Flash Photodynamic Therapy (Flash-PDT)

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The use of pulsed *versus* continuous-wave (CW) lasers was investigated in the early days of PDT and the consensus reached was that pulsed lasers did not offer any significant advantage over CW lasers. It is now very exceptional to find PDT studies with pulsed lasers because CW lasers are more affordable and simpler to use. However, the comparison between pulsed and CW lasers in PDT was empirical rather than based on the design of the best approach for each type of laser. Here, we show that when the use of pulsed and CW lasers is compared using the maximum tolerated doses allowed for each type of laser, rather than compared using the same light dose, pulsed lasers promote safer treatments of larger tumors.

We show that, when photosensitizers with high absorption cross-sections are employed, pulsed lasers saturate absorption over a depth of 1 cm, and can be dosed to obtain a selectivity towards tumor tissue matching the biodistribution of the photosensitizer. The increased selectivity offered by pulsed lasers allows for the use of higher light doses. Using the same laser wavelength, we show that the light dose delivered by pulsed lasers, expressed in J/cm^2, can be increased one order of magnitude relative to the light dose that leads to lethality in mice when CW lasers are employed. The increase the light dose without phototoxicity enabled by pulsed lasers, allows for the delivery of more photons deeper in the tumor tissue. This will change the paradigm of PDT and we name this new modality as Flash-PDT.

Acknowledgments

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Membrane lipids play crucial roles in chlorophyll biosynthesis during chloroplast biogenesis

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Plastids are a diverse family of plant organelles and chloroplasts are the most typical form of plastids that develop the thylakoid membrane inside to perform oxygenic photosynthesis. In angiosperms germinated in the light, proplastids of cotyledon cells differentiate directly to chloroplasts. However, in the dark, proplastids differentiate to etioplasts as precursors of chloroplasts. Etioplasts have lattice membrane structures named prolamellar bodies (PLBs), where protochlorophyllide, a chlorophyll intermediate, accumulates with light-dependent protochlorophyllide reductase (LPOR). With light exposure, etioplasts rapidly differentiate to chloroplasts to establish photoautotrophic growth. The differentiation from etioplasts to chloroplasts involves the dynamic transformation of PLBs to thylakoids, which is accompanied by protochlorophyllide-to-chlorophyll conversion, LPOR degradation, and accumulation of photosynthetic complexes. In contrast to the drastic changes in pigment and protein compositions, the composition of glycerolipids is almost unchanged during PLB-to-thylakoid transformation, suggesting that the lipid molecules in PLBs are directly used for the development of the thylakoid membrane.

The lipid bilayer matrix of PLB and thylakoid membrane mainly consists of four unique lipid classes—monogalactosyldiacylglycerol, digalactosyldiacylglycerol, sulfoquinovosyldiacylglycerol, and phosphatidylglycerol. Our research group revealed that each lipid has specific roles in membrane-associated processes during the development of etioplasts and the differentiation from etioplasts to chloroplasts (1-4). Particularly, phosphatidylglycerol plays an essential role in chlorophyll metabolism and membrane organization in these processes.

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Involvement of a 'super-rogue' photosystem II complex in chlorophyll *f* biosynthesis

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A subset of cyanobacteria has evolved to use far-red light (FRL) to support their photosynthetic growth in a process known as far-red light photoacclimation (FaRLiP)[1]. FaRLiP involves the synthesis and incorporation of FRL-absorbing chlorophyll (Chl) molecules (Chl *f* and Chl *d*) into a new set of FRL-absorbing photosystems to allow oxygenic photosynthesis to be driven by lower energy photons[2].

Bryant and colleagues showed that a divergent D1 paralog found in FaRLiP species, referred to as super-rogue D1[3] (srD1) or ChlF[4], is required for light-induced Chl *f* biosynthesis[4].

We subsequently showed that srD1 expressed heterologously in *Synechocystis* PCC 6803 assembles into a variant PSII complex, termed the super-rogue photosystem II complex (srPSII), that drives Chl *f* synthesis but cannot oxidize water[5].

Here I describe the cryo-EM structure of a His-tagged srPSII complex obtained at a resolution of 2.46Å. The monomeric complex contains the srD1 subunit encoded by *Chroococcidiopsis thermalis* PCC 7203 and 15 of the 17 intrinsic subunits found in oxygen-evolving PSII. Missing are the extrinsic PsbO, PsbU, PsbV and CyanoQ subunits on the luminal side of the complex and the intrinsic PsbJ and PsbY subunits. Present in the srPSII complex are 35 Chls (with 3 predicted to be Chl *f* by HPLC), 10 carotenoids, plastoquinones Q_A and Q_B, the non-heme iron but there is no evidence for the binding of Ca^{2+} or Mn ions. The cryo-EM structure of srPSII reveals altered binding of CP43 to srD1 which might be related to the role of srPSII in Chl *f* biosynthesis. The mechanism is unknown but might involve reactive oxygen species produced by srPSII in the light.

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Biosynthetic pathways for chlorophyll pigments branched by chlorophyllide oxidoreductase and their evolution

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In photosynthetic organisms, chlorophyll (Chl) and bacteriochlorophyll (BChl) are essential for harvesting light energy and transferring electrons. Oxygenic phototrophs such as plants and cyanobacteria utilize Chl molecules, whereas anoxygenic phototrophic bacteria mainly use BChl. A difference in the chemical structures between Chl and BChl occurs at the C7-8 position. Chl molecules have the C7=8 double bond, and in BChl biosynthesis, the double bond is reduced by the enzyme, chlorophyllide oxidoreductase. In other words, the reduction toward C7-8 single-bond formation corresponds to the conversion of chlorin to a bacteriochlorin ring. This conversion provides a large shift in the absorption wavelength by approximately 80 nm. In addition, we found a natural variant of chlorophyllide oxidoreductase that catalyzes the formation of the C-8 ethylidene group as well as C7-8 single bond, resulting in the larger red shift of wavelength by over 100 nm. The variant oxidoreductase causes the committed step toward BChl *b* and *g* biosynthesis. Chlorophyllide oxidoreductase and protochlorophyllide oxidoreductase are similar to nitrogenase in sequence and structure. Construction of a4 phylogenetic tree of nitrogenase-like enzyme family showed that the core subunits of these oxidoreductases arose through sequential gene duplications and form a functionally homogenous phylogenetic group. We analyzed phylogenetic trees of other pigment biosynthetic enzymes and bacterial genomes, and found a clear phylogenetic relationship between the domain Bacteria and photosynthesis. We also conclude that the last phototrophic common ancestor probably had simple pigment biosynthesis proteins including Mg-chelatase, (proto)chlorophyllide oxidoreductase, and (B)Chl synthase.

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Alternative localization of HEME OXYGENASE 1 in plant cells regulates cytosolic heme catabolism

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Heme, an organometallic tetrapyrrole, is widely engaged in oxygen transport, electron delivery, enzymatic reactions, and signal transduction. In plants, it is also involved in photomorphogenesis and photosynthesis. HEME OXYGENASE 1 (HO1) initiates the first committed step in heme catabolism, and it has generally been thought that this reaction takes place in chloroplasts. Here, we show that HO1 in both Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*) has two transcription start sites (TSSs), producing long (*HO1L*) and short (*HO1S*) transcripts. Their products localize to the chloroplast and the cytosol, respectively. During early development or de-etiolation, the *HO1L/HO1S* ratio gradually increases. Light perception via phytochromes and cryptochromes elevates the *HO1L/HO1S* ratio in the whole seedling through the functions of ELONGATED HYPOCOTYL 5 (HY5) and HY5 HOMOLOG (HYH) and through the suppression of DE-ETIOLATED 1 (DET1), CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1), and PHYTOCHROME INTERACTING FACTORs (PIFs). *HO1L* introduction complements the *HO1*-deficient mutant; surprisingly, *HO1S* expression also restores the short hypocotyl phenotype and high pigment content and helps the mutant recover from the *genomes uncoupled* (*gun*) phenotype. This indicates the assembly of functional phytochromes within these lines. Furthermore, our findings support the hypothesis that a mobile heme signal is involved in retrograde signaling from the chloroplast. Altogether, our work clarifies the molecular mechanism of *HO1* TSS regulation and highlights the presence of a cytosolic bypass for heme catabolism in plant cells.

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The role of the carotenoid β2-ring and the N-terminal domain in the OCP photocycle: new insights

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Adaptation to rapid environmental changes is crucial for maintaining optimal photosynthetic efficiency and is ultimately key to the survival of all photosynthetic organisms. In cyanobacteria, the major aquatic primary producers, photoregulation is controlled by the orange carotenoid protein (OCP) photocycle. OCP is the only known photoreceptor that uses a carotenoid for light activation. Understanding and potentially controlling this unique photocycle could open up new opportunities for optogenetics and improving photosynthetic biomass. How the carotenoid drives and controls it remains unclear. It has long been argued that OCP photoactivation is initiated in the C-terminal domain (CTD) by H-bond cleavage between the carbonyl group of the carotenoid β1-ring and adjacent residues. However, my recent crystallographic results suggest that the H-bond cleavage occurs after carotenoid isomerisation and rearrangement of the N-terminal domain (NTD) (ref 1). All this suggests that previous models of the OCP photocycle should be re-evaluated. To better understand the role of the NTD in the photocycle, we have performed temperature-dependent spectroscopy, flash photolysis and pump-probe transient absorption on the two OCP forms: Canthaxanthin-bound

OCP (OCP_{CAN}) and Echinenone-bound OCP (OCP_{ECH}). The difference between the two carotenoids is the presence of a carbonyl group in the β2-ring located in the NTD of the protein. The applied spectroscopic approach allowed us to report the previously unresolved OCP intermediate, mainly associated with the absorption bleach. We show that the steps of the OCP photocycle are always faster in OCP_{CAN} than in OCP_{ECH}: from 2 to almost 20 times, depending on the step. These results suggest that the presence of the carbonyl group in the β2-ring of the carotenoid accelerates the OCP photocycle and that NTD plays an important role in the OCP photocycle, at least in the μs-s time range. An updated model of the OCP photocycle is proposed.

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Current Treatment Interventions for Myopia Progression in Children

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Myopia is predicted to involve 50% of the global population by 2050[1]. Myopia progression in children is due to rapid elongation of the eye. The longer the eye, the higher the myopia, leading to an increased risk of sight-threatening eye diseases in later life[2] [3]. Research and development have increased our understanding and are now providing interventions for this faster-than-normal progression[4] [5]. This talk will explain the underlying condition and the new interventions, which include lifestyle, refractive, pharmacology and specific wavelength light therapy. The use of natural light and specific wavelength light will be expanded in the subsequent talks, highlighting the research, benefits and side effects of the intervention.

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Light-based interventions for myopia prevention and control: from bench to classrooms

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Myopia is a refractive error characterized by the blurred vision of objects viewed at a distance. It is far more than a mere inconvenience and represents a true, highly prevalent, sight-threatening disease, that has reached epidemic proportions in Asia. Besides its high socio-economic burden, high myopia (spherical equivalent of -5 Diopters or worse), affecting 2.7% of the world population, can lead to serious ocular complications and vision loss. The risk of high myopia is particularly high in children with myopia onset during the early school ages, which is common in Singapore. To reduce the risk of high myopia development in adulthood, today there's an urgent need for effective prevention strategies for early-onset myopia in Singapore and Asia.

Increased outdoor time is protective against myopia. Analogously, experimental studies in animal models of myopia and interventional studies in humans suggest that exposure to bright light, similar to daylight, or even brighter light levels indoors, can prevent or delay myopia onset.[1–4] Concomitantly, spectrally-tuned, moderate levels of light (*e.g.,* indoor light levels) can limit myopia development.[5,6]

In this talk, I will share our team's journey to understand the protective features of outdoor time and optimize the spectro-temporal characteristics of light exposure for myopia prevention in several animal models, while exploring underlying mechanisms and technical applications. Additionally, I will introduce our latest school-based trial, "LightSPAN," which aims to translate our findings into improved light exposure for children, providing a more effective, safe, and scalable approach to myopia prevention and control.

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Biomarkers of sun exposure and eye diseases: Pterygium and Myopia (opposite sides of the coin)

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Sun damage to the eye has long been known and the recommendation to add sunglasses to the Australian SunSmart campaign (Slip, Slop, Slap + Seek & Slide) reflected this.

We found that since the SunSmart campaign the number of surgical procedures for Pterygium has declined in Australia.

On the flip slide, the world is seeing an epidemic of myopia particularly in the cities of East Asia. The main risk factors for myopia is lack of time spent outdoors.

With our Australian cohorts (Twins Eye Study in Tasmania & Brisbane, Norfolk Island Eye Study, The Raine Study, Kidskin Study and Busselton Healthy Aging Study) we have been comparing markers of excess sun exposure with Myopia and Pterygium. We have validated Conjunctival UV Autofluorescence area as a biomarker of ocular sun exposure with high levels of CUVAF associated with Pterygium and low levels with Myopia. In Busselton we showed that adults who had skin cancer had half the rate of Myopia (11%) compared to those without skin cancer (22%).

Post COVID-19 data suggests the lack of time outdoors is most important in primary school aged children. Data from the Sydney Myopia Study and trials from China and Taiwan suggest 2 hours outdoors per day is recommended. The challenge is balancing adequate time outdoors with UV protection to minimise Skin Cancer, Pterygium and Myopia.

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Investigating the Effects of Short-term, Supra-threshold Red Laser Light Irradiation on Retinal Structure and Function in Pigmented Rabbits

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Purpose: Low-energy red laser irradiation has been rapidly promoted for clinical use in the prevention and control of myopia in adolescents. However, the safety of this technique's dosage needs further investigation. This study aims to explore the acute pathological characteristics of retinal structural and functional damage induced by supra-dose 650 nm diode red laser irradiation using a pigmented rabbit model.

Methods: 16 eyes from 8 pigmented rabbits were studied, the rabbits were randomly divided into three groups: Experimental group: Right-eye irradiation (n=6); self-control group: Left-eye self-control (n=6); blank control group: No irradiation (n=4). The experimental group received 3 minutes of 650 nm red laser irradiation (20 mW, diode laser lamp) on the right eye of 6 rabbits, while their left eyes served as self-controls. The blank control group consisted of 2 rabbits (4 eyes) with no irradiation. Using optical coherence tomography (OCT) to evaluate retinal structure before irradiation. Changes in the retinal structure and function of the irradiated eyes were observed using electroretinography (ERG) and OCT. Apoptosis of retinal cells was examined by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Expression of p53 and caspase 3 in retinal tissue was measured using immunofluorescence. These assessments were used to analyze the acute damage characteristics of the retina following supra-dosage 650 nm diode red laser irradiation.

Results: After a 3-minute-irradiation with 20 mW red light, ERG results demonstrated significant delays in b-wave、OPS-wave latency ($p < 0.05$) and a reduction in amplitude ($p < 0.05$) for the irradiated eyes under dark adaptation. A significant decrease in a-wave amplitude ($p < 0.05$) was observed under dark adaptation. The analysis of OCT results revealed that the structure of the retina in the irradiated eye was disrupted, characterized by outer retinal layer edema, disruption of the retinal pigment epithelium, and increased reflectivity in the inner retina. More TUNEL-positive apoptotic cells were observed in the outer nuclear layer of the retinal tissue in the experimental group. Higher expression levels of p53 and caspase 3 were detected in the outer nuclear layer of the retinal tissue.

Conclusions: Short-term exposure to supra-threshold red light induces structural disorganization of the retina and compromises retinal electrophysiological function in rabbits. Further investigation into the safety implications of red light therapy is warranted to ensure its clinical applicability.

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Multiconfigurational quantum chemistry to study macromolecular systems in photobiology

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Multiconfigurational quantum chemistry (MQC) is a computational tool useful to study chemical transformations in which several electronic configurations contribute at the same time. Such situations are usually called strong correlation problems. They are very common in excited-state chemistry, induced either by light (photochemistry) or by a chemical reaction (chemiluminescence). MQC is computationally demanding and as a consequence difficult to apply to large macromolecular systems as those of interest in biology (proteins, nucleic acids, etc.) and also in other fields such as nanotechnology or atmospheric chemistry. In this talk, recent advances developed in the framework of the Quantum Chemistry of the Excited State – Universitat de València

(QCEXVAL) group will be presented. They include developments using MQC in macromolecules to determine band lines of absorption and emission spectra of (implemented in MultiSpec and OpenMolcas softwares) and to obtain triplet free energies.

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Multiscale simulations insights into triplet thymine formation and reactivity.

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Triplet thymine is well known as a hotspot for DNA photo damage, and a reference experiments value for its energy has been inferred experimentally [1]. This average value may blur a DNA sequence dependence which can be revealed by ad hoc simulations. Beyond the isolated thymine which has been much studied [2], the dynamics of triplet thymine in a B-helix has been less investigated computationally. We have investigated owing to QM/MM-MD simulations which highlight the interaction mode of DNA-drug for a large range of photosensitizers [3,4] including benzophenone. Based on these structures, we can map the triplet-triplet energy transfer in short oligonucleotides relying on static QM/MM schemes. We also investigate in situ the impact of micro hydration in tuning photoproperties of a luminescent DNA probe [5] relying on non-adiabatic dynamics. In the future, we plan to investigate the action of photosensitizers with nucleosome core particles for which the mechanical and electrostatic embedding differ from B-DNA.

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Light-induced anticancer therapies: A computational perspective

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Light exploitation as a source of selectivity in anticancer therapies is gaining interest in the last years.[1] The use of biocompatible wavelengths requires the administration of photoactive chromophores that must be distributed into cancerous cells in sufficient concentration to trigger clinically relevant photodamage upon irradiation of the ill area. Therefore, efficient photophysical and photochemical processes are a prerequisite for new photosensitizers to be candidates for clinical development. Traditionally, light-induced biological damage is exerted through the classical O_2 mediated type I and/or type II photodynamic therapy (PDT) photoreactions. However, the physiological conditions of solid tumours often imply low levels of molecular dioxygen, limiting the outcome of the already clinically approved drugs.[1]

The present talk will describe recent approaches to circumvent the hypoxia problem from a multidisciplinary perspective, emphasising however the contributions of computational chemistry in the elucidation of the molecular mechanisms behind the photodamage. The photoprocesses that will be considered are mediated by molecules based on heavy transition metals[2,3] and by some metal-free chromophores[4,5] for instance by releasing nitric oxide (NO) upon excitation.[4] I will show recent results on how these non-canonical photosensitizers respond upon light absorption, and how this information reveals the photochemical mechanisms responsible of the biological damage.

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MRSF-TDDFT: A New Quantum Mechanical Workhorse for Photobiology

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A novel quantum theory known as MRSF-TDDFT (Mixed-Reference Spin-Flip Time-Dependent Density Functional Theory)* has been developed to address numerous limitations associated with the widely used DFT and TDDFT theories. This advancement positions MRSF-TDDFT as an excellent alternative for general scientific applications. Notably, MRSF-TDDFT demonstrates promising utility in addressing challenging nonadiabatic processes within biological systems.

This theory offers several theoretical advantages over existing models and has shown impressive performance in recent studies. Specifically, its application to phenomena such as firefly chemiluminescence and the fluorescence of GFP (Green Fluorescent Protein) chromophores exemplifies its practical significance and broad applicability, topics that will be covered in this presentation.

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PHOTOCHECK - Photodynamic Therapy with Checkpoint Inhibition for Elimination of Anal SCC

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Photodynamic therapy to treat cancers promises the opportunity to delivery focused activation leading to the generation of cytotoxic radical oxygen species with an acceptable safety profile. Commensurate with direct tumour cell killing is the collateral stimulation of host immune responses. The ability to deliver photo-activation presents challenges regarding clinical tractability: - which patients to treat, what cancers and the most reasonable path to test agents in patients. We have developed a mouse model for anal carcinogenesis that faithfully recapitulates the human disease including the predominant associated *PIK3CA* gene mutation. Cell lines from these mouse anal squamous cell carcinomas (SCCs) form tumours in immune competent, syngeneic mice. As these tumours grow when injected subcutaneously, they are amendable to the topical application of a novel photodynamic therapy using a next generation compound, INV043. Clinically, SCCs can be divided into human papilloma virus (HPV) positive and negative: - the latter category is the most difficult to treat. The anal SCC model reported here is HPV-ve. When INV043 is used alone some tumour control is evident but when combined with immunotherapy checkpoint-blockade (anti-PD1) 80% of mice eliminate the tumours masses and show no evidence of residual tumour cells. Anti-PD1 alone cured 12% of mice with established tumour. Evidence of superior immune cell recruitment with anti-PD1 combined with INV043 led to complete, scar-free tumour resolution. Based on these data we propose to develop a Phase 1 safety trial (PHOTOCHECK) to treat patients with relapse anal SCCs prior to salvage surgery. Ten patients (HPV+/-) would be recruited over 2 years. A pretreatment biopsy will be taken prior to two rounds of photodynamic therapy (over 3-days) and two rounds (3-weeks apart) of anti-PD1 antibody. Tumours will be monitored for one month prior to surgery. This plan allows an assessment of pathological responses along with immunological changes.

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Integrating plant fluorescence, photosynthesis dynamics and the surveillance of chemical risk in the environment

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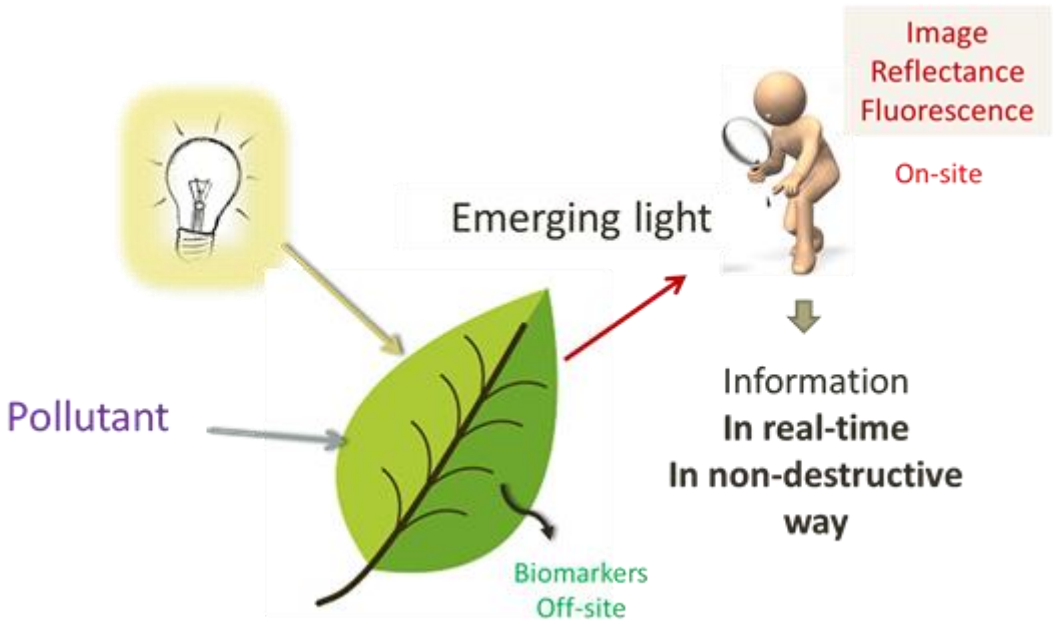
In this talk, I will explore the combination of rapid *in situ* photochemical early warning methodologies with specific biomarker-based methods and off-site determinations for the surveillance of chemical risk in the environment.

I will highlight how chlorophyll fluorescence can be used as an effective tool for early detection of contaminants in the environment. These techniques, being rapid and non-destructive, offer the advantage of being performed directly in the field, alerting us to the presence of pollutants before identifying their specific chemical nature. The starting hypotheses are the following: the toxic chemicals affect the photosynthesis dynamics and pigment content of plants. Changes in photosynthesis efficiency will lead to modifications in chlorophyll fluorescence, whereas variations in pigment concentration will affect the plant reflectance. Since alterations in spectroscopic or optical signals emerging from vegetation, may be easily observed in non-destructive way, they offer a convenient and non-invasive tool to rapidly detect disturbances in plant physiology due to pollutants.



In addition, I will discuss how these early warning techniques can be complemented with the determination of contaminant-specific biomarkers by performing more detailed analyses in the laboratory. I will present concrete examples of plant systems designed to detect the presence of organophosphates, known acetylcholinesterase inhibitors, in the environment. These pesticides affect the central nervous system of animals, and the implementation of natural plants as sentinels could provide essential early warning.

I will show how optical methodologies can be complemented with specific and novel electroanalytical methods to quantify the toxic chemicals through the quantification of Acetylcholinesterase inactivation.

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Microplastics and solar UV radiation

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Abstract content TBC

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Biocatalytic production of solar chemicals by photosynthetic microbes

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Photosynthetic microorganisms, such as microalgae and cyanobacteria, have immense potential for fossil-free production of desired compounds and can play a crucial role in sustainable biotechnologies. In contrast to conventional biorefinery approaches applied to microalgal biomass, biocatalytic production with engineered photosynthetic microbes involves the production of targeted chemicals using light energy, which are then secreted, enabling a "milking" process. We explore two distinct approaches to biocatalytic production. In the first approach, photosynthetic cells serve as whole-cell catalysts, converting introduced chemicals (e.g. cyclohexanone) into desired products (e.g. ε-caprolactone) by utilizing photosynthetically produced molecular oxygen and reducing equivivalents such as NADPH. This approach allows for sustainable cofactor regeneration through photosynthesis, overcoming a major challenge in biocatalytic production.

In the second approach, we transform suspension cultures into photosynthetic engineered living materials (hydrogels) by encapsulating cells within an environmentally friendly polymeric scaffold matrix. This method ensures a safe and controlled environment for the cells, addressing various challenges associated with suspension cultures and enabling long-term production (several months). In case studies, 3D-printed cyanobacterial and microalgal cells, entrapped within thin hydrogel layers using photocurable polymers, exhibited notably high production titers and space–time yields compared to conventional biocatalysts. These photosynthetic engineered living materials, which are biocompatible and derived from renewable resources, particularly when integrated with 3D printing, offer scalability and the potential to enhance sustainability in the chemical industry.

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SynBio strategies in photoautotrophs for improved carbon fixation, growth, and yield

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Current anthropogenic CO_2 emissions have reached record levels while the demand for energy, food, and materials continues to climb. Fortunately, our planet receives from the sun more energy than is needed to support all human activities. Photosynthetic organisms like cyanobacteria and plants can efficiently capture solar energy to convert inorganic carbon into organic molecules. However, synthetic biology approaches in photoautotrophs remain underdeveloped. This talk explores different strategies to improve carbon fixation, growth, and yield in these organisms. The first strategy uses barcoded CRISPRi libraries coupled with growth-coupled screening to identify genes whose repression enhances growth. The second approach uses mass spectrometry techniques to identify novel protein-protein (BioID) and protein-metabolite (PISA and Lip-Smap) interactions, providing insights into metabolic regulation and serving as potential targets for mutagenesis to create unregulated variants. A third strategy uses generative AI to design new enzyme variants with improved properties. These resulting proteins are then screened *in vivo* in a cyanobacteria platform for enhanced growth, and the optimized genes are subsequently transferred to bioproducing strains or plants to boost productivity. This combination of systems biology, mutagenesis, and metabolic engineering approaches holds promise for engineering novel metabolic modules for increased growth, and these results could have broad impacts on bioproduction, from chemical manufacturing to agricultural yields.

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Engineering solar lipid production in oleaginous microalgae: a focus on the prospects and challenges on the path to optimization and industrialization.

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Over the past seven decades, microalgae have garnered attention as promising candidates for industrial exploitation in food and biofuels due to their high productivity, versatility in growing in fresh and seawater, and independence from fertile land[1]. Harnessing their potential for producing proteins, hydrocarbons, and fatty acids holds promising prospects for various industries. However, to ensure economic viability, optimization in solar energy conversion, carbon capture, and metabolic flux partitioning is imperative[2-3]. Our analyses showed that solar to lipid conversion is still one of the major causes of the high costs of bulk lipid production[4-5]. So, how do we optimize this process? Here we report some considerations and some strategies that we perform, aimed at increasing the lipid productivities, by taking advantage of the natural genetic variability, by inducing mutations in a parental population, by targeted genetic engineering[5,6]. Our recent research focuses on genetically domesticating robust, oleaginous marine microalgae, particularly *Nannochloropsis oceanica*, known for its high triacylglycerides (TAGs) and omega-3 eicosapentaenoic acid (EPA) content. Strategies such as genetic engineering, induced mutations, and leveraging natural genetic variability are being employed to enhance lipid productivities[3,7]. This talk provides insights on the developed cutting-edge tools like CRISPR-Cas systems for precise gene editing and the exceptional high-gene expression system based on RNA polymerase I activity[8-10]. Additionally, we will discuss successful applications of high-throughput screening techniques to identify and select new mutant lines with increased lipid phenotype[11-14]. Moreover, we discuss the targeted genetic engineering efforts to tailor the lipid composition of N. oceanica for specific applications, such as replacing tropical oils and incorporating novel lipid classes like medium-chain fatty acids[15]. Overall, this forward-thinking approach underscores the significant role microalgae could play in sustainable industrial applications. are innovative strategies that could significantly impact various industries.

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Exploring the Marvels of Anoxic Photosynthesis for Revolutionary Agri-Energy Production

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In view of the increasing demand for biofuels and sustainable chemical feedstocks, we develop a new approach in agriculture, aiming to harness photosynthetic microorganisms such as microalgae and cyanobacteria. In stark contrast to land plants, these microbes can be harvested daily. Furthermore, the advanced genetic tools gained so far for these microbes, which gave one microalgae species, *Chlamydomonas reinhardtii* the unique name "the green yeast" allow reengineering into efficient green chemistry reactors for sustainable and clean production of chemicals and fuels. While photosynthetic energy, channeled through electron flow, is primarily used to power the fixation of CO_2 into organic matter, it can also power other limited processes, such as H_2 production via the enzyme Hydrogenase.

Photobiological Hydrogen (H_2) production from green microalgae holds great promise for sustainable production of a clean, zero carbon footprint fuel. However, in nature, the process of H_2 production is temporary, lasting for 2 minutes. Thereafter, it ceases due to electron loss to competing processes, mainly the Calvin cycle, and later on, due to an accumulation of inhibitory concentrations of oxygen. The key for diverting the energy from CO_2 fixation to H_2 production or other chemicals is to unveil and exploit new photosynthetic hotspots, where electron flow can be redirected towards improved enzymes. Recently, we found that a *Chlamydomonas* mutant in the Proton-Gradient-Regulation-Protein-5 (*pgr5*) gene harbors faster respiration and a slower Calvin cycle allowing scalable (culture volumes of 1L) continuous production of H_2 under ambient mixotrophic conditions for a duration of 12 days. This achievement allows engineering studies which focus on scale up of the process.

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Current status and future opportunities in PDT

Colin Hopper[1]

1. Invited , Speaker

Since the first description of photodynamic therapy at the beginning of the last century there was an initial interest in antimicrobial treatment that was lost following Fleming's discovery of penicillin. Subsequent development was focused on the treatment and mapping of cancer and this remains a major focus. However the emergence of antibiotic resistant bacteria has caused us to revisit light activated antimicrobial treatments.

There are currently very few licensed drugs for PDT and most of the current clinical applications are focused on three drugs – the Photofrin ALA and Foscan and clinical examples will be shown to highlight the use of these albeit with a predominant focus on applications in the head and neck.

More recent studies have focused on the synergy of PDT with chemotherapy and immunotherapy as well as photochemical Internalisation and these will be outlined wherever possible (some details will be excluded as a result of a IP issues). There has also been an increase in activity in the use of PDT in low to middle income countries and I will give examples of these.

Lastly the increasing interest in antimicrobial PDT will be addressed as this is showing great promise in surgical site infection reduction.

In this presentation I will give an overview of current indications for PDT and opportunities for further research.

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Fluorescence-guided resection of brain tumours with ALA

Kate Drummond[1]
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ABSTRACT CONTENT TBC

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Photoacoustic imaging and photodynamic therapy efficacy of polyacrylamide and gold nanoparticles containing near infrared photosensitizers

Ravindra Pandey[1]
1. Roswell Park Comprehensive Cencer Center, Buffalo NY
Abstract content TBC

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Modification of extracorporeal photopheresis with 5-aminolevulinic acid (Gliolan)

Qian Peng[1]
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Extracorporeal photopheresis (ECP), a modality that exposes isolated white blood cells to photoactivatable 8-methoxypsoralen and UVA light ex vivo followed by returning the treated leukocytes to the body, is used for the treatment of cutaneous T cell lymphoma, graft versus host disease and some other T-cell-mediated diseases. However, the disadvantages of this therapy include the destruction of both diseased and normal T cells with little selectivity, and clinically, long-lasting, expensive and only partial response in the majority of treated patients. Furthermore, the mechanism of action is not fully understood, so that it makes difficult to broaden application to additional types of T-cell-mediated diseases. Selective, short duration, cheap and more effective alternatives are thus needed. Our previous studies over a 30-year period have established a broad biological basis for introducing a new concept of ECP technology with the potent photosensitizer protoporphyrin IX derived from its precursor, 5-aminolevulinic acid (ALA) (Gliolan, photonamic, GmbH & Co. KG, Germany). The use of ALA for ECP may cause selective and effective immunogenic cell death of proliferative malignant or activated T-cells without compromising functions of the normal T-cells to induce systemic anti-disease immunity. Our ongoing preclinical and clinical studies will be presented.

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Sunlight oxidative impact in xeroderma pigmentosum variant mutagenesis and tumors

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Human xeroderma pigmentosum variant cells are deficient in pol eta (*XP-V/POLH* gene), affecting translesion synthesis following solar ultraviolet (UV) light-induced DNA damage. Consequently, these patients have a high frequency of skin tumors in regions exposed to sunlight. We investigated how XP-V cells respond to UVA light, corresponding to the most intense solar UV to reach patients. These cells are more sensitive to UVA light when compared to control cells, but interestingly, an oxidative stress effect is induced in these cells, inhibiting the repair capacity of these cells. The XP-V cells present both replication and cell cycle block completely protected in cells pre-treated with N-acetyl cysteine antioxidant agent. Mutagenesis in these cells through whole-exome sequencing also revealed that UVA-irradiation increase in C>T transitions, mainly at potential pyrimidine dimer sites, but also a substantial contribution of C>A transversions, potentially due to oxidized bases, even in XP-V cells V non-irradiated. Interestingly, the mutation profile of skin tumors from XP-V patients also discloses strong participation of C>A mutations in a particular sequence context. Moreover, some tumors revealed a mutation signature related to tobacco chewing. The results indicate that oxidation may be responsible for at least part of the phenotype in XP-V patients. Thus, including antioxidant protection in the everyday life of XP-V patients may contribute to the improvement of their life quality. Interestingly, XP-V skin tumors also presented a high frequency of retrotransposition insertion, compared to skin tumors from normal population. We are now investigating the mechanisms responsible for these transposition events, as the increase in single stranded DNA observed in XP-V cells replicating their damaged DNA may provide a substrate for retrotransposition.
Financial Support: FAPESP, CNPq, CAPES.

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Novel insights about melanoma response after melanogenesis stimulation and phototherapy

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Melanomas are tumors derived from melanocytes, which have high mortality and low treatment efficiency. Melanocytes are cells that produce the pigment melanin, which, under normal conditions, have the function of protecting the cell from possible damage caused by UV radiation, both by absorbing part of this radiation and by sequestering reactive oxygen species (ROS). However, many studies have shown that melanin in tumor cells may be related to processes of resistance to conventional treatments, especially photodynamic therapy. The process of melanin production (melanogenesis) in melanoma cells leads to a transient increase in ROS, which consequently induces several cellular modifications that allow the maintenance of cellular viability. Many studies on the effects generated by ROS in melanoma cells have been carried out, but little is known about the profile of proteins that undergo redox modification and that can act as signals in tumor survival. We investigated the profile of redox-modified

proteins in murine melanoma cells with stimulated melanogenesis and submitted to rose bengal-photodynamic therapy (RB-PDT). A redox proteomics label-free approach based on the biotin switch assay technique with biotin-HPDP and N-ethylmaleimide (NEM) was optimized and used to assess the thiol-oxidized protein profile. Our results showed alterations that may affect biological processes, such as calcium signaling, translation, signal transduction, energetic metabolism, genomic stability, protein folding and trafficking, and stress response via redox signaling. The redox alterations observed in melanoma cells and identification of possible target proteins are of great importance to further understand tumor resistance mechanisms.

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The role of UVA and UVB induced DNA damage and mutations in melanoma

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Melanoma is a lethal type of skin tumor that has been linked with sunlight exposure. Wavelengths from the sun that can reach the earth's surface include UVA radiation (320-400 nm) and UVB radiation (280-320 nm). UVB effectively induces the formation of dimeric DNA photoproducts, preferentially the cyclobutane pyrimidine dimers (CPDs). The characteristic UVB signature mutations in the form of C to T mutations at dipyrimidine sequences are prevalent in melanoma tumor genomes and can be ascribed to deamination of cytosines within CPDs before DNA polymerase bypass. However, evidence from epidemiological, animal, and other experimental studies, although somewhat controversial, also suggest that UVA radiation may participate in melanoma formation. The DNA damage relevant for UVA includes specific types of CPDs at TT sequences and perhaps oxidative DNA damage to guanine. We have been applying sensitive methodologies for genome-wide mapping of different types of DNA damage in human skin cells and have been using these approaches for linking UV-induced DNA damage and mutations to melanoma-specific mutational signatures.

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Genome-wide studies of nucleotide excision and photolyase repair mechanisms for UV damage in yeast

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UV light damages DNA by inducing cyclobutane pyrimidine dimers (CPDs) and other lesions that must be efficiently repaired to avoid cell death or mutagenesis. While essentially all species can repair CPDs via nucleotide excision repair (NER), many species, including bacteria, yeast, and other eukaryotes, also utilize photolyase enzymes to repair UV damage. How these disparate repair pathways function in concert to repair UV damage across a eukaryotic genome packaged in chromatin remains unclear. Here, we use our CPD-seq method to map repair of CPD lesions by NER and photolyase across the yeast genome. Our data indicate that NER repairs damage throughout the genome, and that this repair is modulated by chromatin and histone acetylation. CPD lesions are more rapidly repaired by yeast photolyase, but this repair is significantly inhibited when damage is located in certain classes of transcription factor binding sites or in nucleosomes. Repair of damage in nucleosomes is particularly inhibited when CPDs are located at the 3' side of the nucleosomal DNA or at minor-in rotational settings. While photolyase efficiently repairs the non-transcribed strand (NTS) of yeast genes, repair of the transcribed strand (TS) is inhibited. Genome-wide analysis of UV-induced mutations in NER-deficient, photoreactivated yeast revealed a striking enrichment of mutations along the TS of yeast genes. Taken together, these data indicate that inhibition of photolyase repair along the TS, likely due to occlusion of CPDs by RNA polymerase II stalling, promotes UV mutagenesis.

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Genome-wide impact of cytosine methylation on UV-induced damage formation

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The integrity of DNA is constantly attacked by a wide array of exogenous and endogenous mutagens, one of the most ubiquitous being UV exposure from the sun. UV induces DNA damage, primarily cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine-pyrimidone photoproducts (6-4PPs), that inhibit transcription and replication and can cause mutations. Although UV mutagenesis has been extensively studied, there have been several conflicting reports regarding the impact of cytosine methylation (i.e., 5-methylcytosine) on the induction of UV damage. We hypothesized that the presence of cytosine methylation will promote the formation of CPDs following UVB irradiation in naked yeast genomic DNA. To test this, we examined the impact of cytosine methylation on UV damage formation using a genome-wide and single-nucleotide resolution mapping method known as CPD-seq developed in our lab. CPD-seq was used to map CPDs in UV-irradiated yeast genomic DNA in the presence and absence of cytosine methylation at CpG sites by purified *M. SssI* CpG methyltransferase. This analysis revealed an average ~2-fold induction of CPD formation in methylated genomic DNA following UVB irradiation as compared to non-methylated controls. We also identified that the magnitude of this induction was dependent on the flanking DNA sequence context. Sequence contexts with a higher propensity to form CPDs in the absence of cytosine methylation showed a weaker CPD induction following methylation. These findings reveal the effects of cytosine methylation on UV damage formation across a eukaryotic genome.

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The effect of UV-induced *Cdkn2a/p16* promoter mutations on the binding of ETS transcription factors

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Skin cancer is strongly associated with ultraviolet (UV) radiation that generates many mutations. There is thus a need to investigate what mutations drive skin cancer. Silencing of the *CDKN2A* gene, which encodes the p16 tumor suppressor protein, plays a key role in cancer progression. Our previous mouse study showed that chronic UV irradiation to skin induced mutations in the regulatory and gene body regions of tumor suppressor genes, including the *Cdkn2a/p16* promoter. Importantly, topical application of caffeine to mouse back skin reduced the frequency of these *Cdkn2a/p16* promoter mutations and suppressed skin cancer development. However, the impact of these promoter mutations on cancer progression remained unclear. We hypothesized that these mutations may inhibit the binding of critical transcription factors, thereby reducing the expression of the p16 tumor suppressor. The mutations at the *Cdkn2a/p16* promoter were found in the DNA sequence that was similar to the ETS transcription factor-binding element (EBE). To determine the functionality of this DNA sequence at the *Cdkn2a/p16* promoter (p16-EBE), we cloned this putative EBE into the reporter construct in which the binding of transcription factors to p16-EBE drives luciferase expression. ETS1 and ETS2 represent the ETS transcription factor family, with the latter expressed dominantly in skin. We found that overexpression of either mouse ETS1 or ETS2 resulted in higher luciferase activities than no overexpression control, indicating that ETS transcription factors bind to p16-EBE. We also tested mutated p16-EBE that carries the UV-induced mutation found in mice. With overexpression of mouse ETS1 or ETS2, the mutated p16-EBE showed markedly lower luciferase activities than wild-type counterpart, implying that the UV-induced mutation in p16-EBE inhibits the binding of ETS proteins to the *Cdkn2a/p16* promoter. This study highlights the importance of loss-of-function mutations in promoters that may contribute to cancer progression.

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Effect of dose-delivery and exposed area on thymidine dimer excretion in urine. -A study in healthy volunteers

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Introduction

Exposure of skin to solar ultraviolet radiation (UVR) induces the formation of thymidine dimers in DNA, serving as a biomarker for UVR exposure. Traditional analysis methods, like 32P-postlabeling, have been replaced by simpler LC-MS/MS techniques (1,2).

Aims

In this study, the impact of dose-delivery and exposed area on thymidine dimer excretion was investigated. **Part 1** sought to determine if the administration of UVR dose influences thymidine dimer excretion by comparing the excretion after a single dose of 3 standard erythema doses (SED) with three doses of 1 SED given on consecutive days. **Part 2** aimed to assess how the excretion of thymidine dimers is affected by the amount of clothing worn during exposure to 2 SED on 3 consecutive days.

Methods

Volunteers (n=16, part 1), (n=30, part 2) were exposed to UV6 tubes emitting 66% UVA and 34% UVB radiation. Morning urine samples were collected daily up to 8 days post-exposure and analyzed using LC-MS/MS. In **Part 1**, all volunteers were full body exposed. In **Part 2**, volunteers were divided into three groups with approximately 75%, 60%, or 45% exposed body surface area. Pigmentation and erythema values were measured using skin reflectance.

Results and Discussion

In **Part 1** significantly higher levels of thymidine dimers were found in the group receiving a single dose of 3 SED compared to those receiving three doses of 1 SED (median 4325 ng vs. 730 ng, p=0.001). In **Part 2**, significant correlations were observed between thymidine dimers in urine and body surface area exposed (r^2=0.20, p=0.012), as well as thymidine dimers and change in erythema (r^2=0.38, p=0.00026).

Conclusion

In conclusion, receiving a single high dose of UVR results in higher thymidine dimer excretion compared to multiple lower doses. Additionally, there is a correlation between body surface area and excreted thymidine dimers in urine.

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State-of-the-Art and Perspectives for Nanomaterials Combined with Nitric Oxide Donors for Biomedical Applications

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The free radical nitric oxide (NO) is a signaling molecule that controls several important physiological and pathophysiological processes in mammals. In humans, the biological impacts of NO include, but are not limited to, key roles in cardiovascular, neurological, immunological, respiratory, and reproductive systems. Depending on its concentration, location and cellular environment, NO can have protective or toxic effects. As NO is a free radical, several classes of NO donors/generators have been prepared and combined with nanomaterials, in particular, with polymeric nanoparticles. Engineered nanoparticles are attractive nanocarriers extensively used in biomedical applications, particularly, in cancer biology due to their ability to promote a site-target therapeutic effect, with minimum side effects to health tissues. NO-releasing nanoparticles can have direct toxic effects on tumor cells, or it can promote cancer cell sensitization for traditional cancer treatments. The combination of NO-releasing nanoparticles with conventional anticancer therapies is a promising approach in the reversion of multidrug resistance (MDR) cells. This work presents and discusses the recent progress in the cytotoxicity (tumoral and non-tumoral cell lines) of NO-releasing polymeric and/or polymer-coated nanomaterials and the in vivo biocompatibility of NO-releasing nanoparticles. Moreover, the ability of these nanoparticles to combat MDR, their mechanisms of toxicity and drawbacks are also discussed. The advantages, challenges, and drawbacks of this strategy are discussed in light of inspiring research on this exciting topic, aiming to translate these innovations into clinical/practical settings.

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Molecular and supramolecular constructs for combined PDT and NO-PDT

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Combination of photodynamic therapy (PDT) with other treatment modalities is emerging as one of the most suited strategy to increase the effectiveness of the therapeutic action on cancer and bacterial diseases and to minimize side effects. In view of the key role nitric oxide (NO) plays in cancer and bacterial diseases, the coupling of PDT with photocontrolled release of nitric oxide (NO) through the appropriate assembling of PDT photosensitizers (PSs) and NO photodonors (NOPDs) may open intriguing horizons towards new and still underexplored multimodal therapies not based on “conventional” drugs and entirely controlled by light stimuli. In this contribution, we present an overview of the most recent advances in this field, illustrating several strategies to assemble PSs and NOPDs allowing them to operate independently without reciprocal interferences and describing the potential applications with particular emphasis to their impact in anticancer and antibacterial research.

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Nitric oxide derivative ruthenium compounds as prototypes for Photodynamic Therapy and X-ray Photodynamic Therapy with low radiation doses

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Cancer is one of the leading causes of death worldwide. Contemporary therapies do not bring the expected effectiveness and the treatment is often non-selective and its application is associated with several side effects significant reducing the quality of life of patients underwent surgery and their long and expensive hospitalization. Photodynamic therapy (PDT) appears as suitable clinical treatment once is non-invasive technique and uses a photosensitizer (PS), oxygen and light irradiation to kill cancer cells. Perhaps the main limitation of PDT is oxygen once tumor is mainly hypoxic. In this lecture, we propose the use of nitric oxide derivative ruthenium compounds in anticancer therapy essentially using basic principles of Photodynamic Therapy (PDT) and X-PDT. The evaluation of photocytotoxic effects is described as a function of structure activity relationship as well the synergistic effect between nitric oxide and reactive oxygen species (ROS) generated by light irradiation in the therapeutic window or X-ray induced PDT with low radiation doses.

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Tailoring nanocarriers to empower the therapeutic potential of photosensitizers

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Biocompatible nanocarriers hold significant potential as versatile systems for delivering photosensitizers (PSs) in therapeutical applications. Their ability to address solubility and stability issues of lipophilic PSs, carry combinations of PSs and other therapeutic agents to a target site, and improve biodistribution following intravenous injection while reducing side effects make them an attractive option for advancing photodynamic therapy (PDT) across various applications. To achieve these potential benefits as a viable therapeutic option, the design and optimization of nanocarriers, including material selection and comprehensive photochemical and technological characterization, are crucial. This presentation will provide a brief overview of the critical steps in designing nanocarriers for PS delivery and then show examples of nanocarriers tailored for combining PDT and chemotherapy and treating bacterial infections. Additionally, the integration of nanocarriers in macroscale platforms such as hydrogels will be discussed as a strategy for the local delivery of PSs.

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Sunheat, water content and temperature as the main drivers of carbon uptake capacity of mosses

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Our recent results in Antarctica (Perera-Castro *et al*. 2020 Frontiers, 2021 JXB) highlights the effect of water availability not only on CO_2 diffusion and photosynthetic capacity, but also on the temperatures experienced by a moss under sunlight due to the high specific heat capacity of water. How both photosynthesis and respiration respond to the interaction of temperature, radiation and water availability is crucial to predict the carbon balance of mosses in different scenarios of climate change. A first approach was done for *Sphagnum* species inhabiting the peatlands near Hyytiälä Forest Research Station (SMEAR II), Finland. Measurements of moss surface temperature under different hydric conditions was done during two weeks, covering different natural sun exposure. In addition, gas exchange at different temperatures and levels of moss hydration was measured for modeling carbon balance. Our results define the effects of different climate change scenarios on peatlands and establish the threshold for day and night temperature above which the existence of this ecosystem could be compromise.

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Ultraviolet photoprotection in Antarctic mosses and liverworts

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Whilst light is essential for photosynthesis and development of plants, both excess photosynthetically active radiation and certain wavelengths (e.g. high energy ultraviolet-B) radiation can be damaging. In mid-summer high levels of photosynthetically active radiation, naturally brings higher levels of UV radiation. However, reduction of stratospheric ozone directly above Antarctica presents another challenge for plants living in the unique Antarctic climate. Depletion of the ozone layer reduces its effectiveness as a UV filter. This subjects bryophytes, the dominant plant life in Antarctica, to elevated and harmful doses of UV-B radiation; levels of which can impair vital cellular contents and processes, such as DNA, chlorophyll and photosynthesis.

Given the high stress environments that many bryophytes inhabit, from hot or frozen deserts to alpine habitats with high incident UV-B radiation, it is unsurprising that they have a suite of photoprotective strategies. Whilst bryophytes share many of these strategies with vascular plants, there are key differences in what is available to bryophytes. Some of these differences pertain to structural features, such as protective epidermal layers, that are available to vascular plants but not generally to bryophytes. Bryophytes thus have to invest more in cellular level photoprotection than vascular plants.

This talk will explore the various mechanisms Antarctic bryophytes (mosses and liverworts) employ to survive under elevated UV-B radiation in an icy desert. This includes the accumulation of specialised compounds called UV-absorbing or -screening compounds that directly or indirectly protect them from UV-induced damage. These often complex sunscreens are highly valuable for many mosses and liverworts surviving the harsh Antarctic environment.

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A perspective on photodynamic activation

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In the past two decades there has been very significant progress in Photodynamic Therapy (PDT). These advancements have come mostly in the form of new concepts based on PDT and new technologies. Examples of concepts adapted from the PDT process are photochemical internalization, photoimmunotherapy, photodynamic priming, radiation-PDT and more. In terms of technology, examples include advanced imaging, biological models, nanotechnology and an emphasis on low-cost PDT.

This presentation will attempt to gather data from different labs to present updates on several of these aspects and put into perspective how these can be used to move the field forward. A discussive format is preferred and participation from the audience will be appreciated to the extent allowed by the allocated time.

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Impact of visible light on human skin

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Visible light (VL, 400-700 nm) was previously reported to have no photobiologic effects on the skin. However, recent studies have demonstrated that it can induce relatively more intense and longer lasting pigmentation, compared to ultraviolet A1 (UVA1, 340-400 nm), in dark-skinned individuals (skin phototypes IV-VI).[1] Additionally, these effects of VL were shown to be potentiated by long wavelength UVA1 (370-400 nm).[2] Subsequent studies also demonstrated that the combination of VL and UVA1 (VL+UVA1, 370-700nm) was able to induce erythema in light-skinned individuals (skin phototypes I-III).[3] Although biologic effects of VL have been established, there is lack of standardized testing guidelines to evaluate photoprotective efficacy of products against this part of sunlight. This invited presentation will discuss the evolution of knowledge of photobiologic effects of VL, associated phototesting methodologies, and available means of VL photoprotection.

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Optogenetic approaches for restoring vision: Where are we now?

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Inherited retinal diseases (IRDs) affect 1 in 3000-4000 individuals globally and is now believed to be the major cause of blindness in developed countries. The FDA approvals of the Argus® II electronic retinal implant in 2013 and the Luxturna® gene-replacement therapy in 2017 have sparked hope for patients with IRDs. Unfortunately, the former has been discontinued due to various reasons including medical complications, while the latter is only eligible to 1 in 100,000 – 2 millions patients with recessive RPE65-linked Leber's congenital amaurosis and genetic correction needs to be performed early in the disease before development of vision-impairing pathology. With over 300 genetic mutations implicated in IRDs, there is a clear need for the development of causative gene-agnostic therapeutic approaches. Optogenetics represents one such approach that has been ardently investigated over the last decade.

Optogenetics for vision restoration involves delivering light-sensing opsins to naturally non-light sensitive inner retinal neurons that remain intact in IRDs to render them light-sensitive, as a means to replace lost or dysfunctional photoreceptors that cause irreversible blindness. There are two major classes of opsins commonly investigated in the field: Type I (microbial) and Type II (mammalian) opsins. Preclinical studies in animal models involving both types of opsins have suggested that optogenetics may restore vision in blind animals. Our preclinical study exploring bReaChES, a red-shifted Type I channelrhodopsin has further suggested vision restoration at ecologically relevant light levels. bReaChES-expressing retinal ganglion cells demonstrate spectral and temporal response characteristics approaching those of normal human photopic vision. The PIONEER phase I/IIa clinical trial that involves delivery of channelrhodopsin, ChrimsonR to retinal ganglion cells, paired with light-stimulating goggles, has reported partial recovery of vision in a blind patient with advanced retinitis pigmentosa.

There is confidence that optogenetics holds promise for restoring vision in IRDs even in late disease stages, but important areas, such as the most ideal candidate, most efficient vector or promoter for gene delivery and expression, and chronic immunogenicity, remain to be investigated to overcome translational challenges.

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Reversing the carcinogenesis-enhancing effects of tacrolimus following exposure to UVB light

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Squamous cell carcinoma (SCC) occurs 65 to 250 times more frequently in solid organ transplant patients than in the general population. These patients must receive life-long immunosuppressive treatment with calcineurin inhibitors, mainly Cyclosporin A or tacrolimus, to avoid organ rejection. Thus, immunosuppression has been widely assumed to be the cause of the higher SCC incidence in these patients. However, recent evidence suggests that calcineurin inhibitors might act directly to induce keratinocyte tumorigenic transformation in an ultraviolet (UV) light-dependent manner by impairing nucleotide excision repair gene expression. Although tacrolimus is more widely used than Cyclosporin A, most studies have focused on the latter, with no published data

showing the in vivo effects of tacrolimus on skin tumorigenesis. Here, by using an in vitro model, we aim to investigate the potential of Q-2361, a tacrolimus antagonist, to reverse the carcinogenic effect of tacrolimus in UVB-irradiated human keratinocytes. We will characterize the impact of both tacrolimus and Q-2361 in keratinocyte and SCC cell survival, proliferation, DNA damage and pro-inflammatory cytokine expression upon UVB treatment. Moreover, we will investigate the influence of tacrolimus and Q-2361 on DNA repair gene and cytokine expression in vivo in mouse ear skin after exposure to UVB. Finally, we will further investigate the protective effects of local Q-2361 treatment against skin carcinogenesis in tacrolimus-treated, immunocompromised mice.

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A new light-adaptive lens improves vision in challenging and varying light situations

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To investigate the influence of a new ultra-fast light-adaptive spectacle lens on glare discomfort (GD), photostress recovery time (PRT) and on dynamic contrast sensitivity recovery in healthy subjects, in challenging or varying light conditions.

Two separate studies were conducted, one in Spain, one in USA, both using a double-blind cross-over randomized design, comparing the new type of light-adaptive lens [TO_1] with a static clear lens. *Study 1* - Ten healthy subjects (average age 28.7 ± 3.4 years) with a spherical equivalent of -1.8 ± 1.8 D (astigmatism < 1D) were pre-trained to control the contrast of a sinusoidal grating with a spatial frequency of 24 cycles/degree placed at 3 m. TO_1 was compared to a clear lens and to a usual photochromic lens TO_2. Subjects were randomly and monocularly exposed to a bright light stimulus (8000 lux) for 30 seconds with either the clear lens, or with the activating TO_1 or TO_2. Then, subjects were transitioned to a dark environment, and contrast sensitivity measures started. The time to recover the first contrast perception after bright light was recorded for each tested lens. Subjects were then searching for their contrast sensitivity threshold dynamically for 100 seconds. Each lens was tested three times. The time required to get the first contrast perception after bright light exposure and the total time required to achieve 80% contrast improvement were computed. *Study 2* - 30 participants (M = 19.2 ±1.3 years) were tested. GD was assessed physically, by measuring palpebral fissure size. PRT was measured as the amount of time necessary to regain visual function after an intense (~15% bleach) 5- second exposure to a broadband (emulated sunlight) light stressor.

Study 1 - There was a significant difference in contrast improvement among the three lenses ($p<0.001$). In dynamic light to dark conditions, TO_1 was 39% faster than clear lens ($p<0.01$) to recover 80% contrast sensitivity, and 23% faster than TO_2 ($p<0.01$). The time to recover the first contrast perception was 55% faster with TO_1 compared to the clear lens ($p<0.001$). The difference between TO_2 and clear lens was not significant ($p=0.03$). *Study 2* - GD was halved ($p < 0.001$) when participants viewed the stimulus through TO_1 compared to the clear control lens. PRT was also recuced by 40% when using TO_1 ($p < 0.001$).

The results highlight the potential of ultra-fast light-adaptive lenses to enhance visual comfort and performance in challenging and varying lighting conditions, compared to the traditional static clear lenses. This improved performance, minimize stress and enhances visual function under normal viewing conditions.

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The Effect of Photodynamic Therapy on Prostate Cancer.

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Scott Carpenter4, Michael Cho4, Hon Cho4, Sebastian Marcuccio3, Donald Murphy4 & Daniel Garama1,2.

Prostate cancer is the most common form of internal cancer in Australian men, with 1 in 6 diagnosed before the age of 85 and 3000 men dying from the disease each year. Early detection and more informed treatments can significantly improve men's prostate cancer survival and research is imperative to understanding this cancer and developing 21st century therapies for this disease. Photodynamic therapy (PDT) uses a combination of a photosensitiser (PS) and light of a specific wavelength, generating reactive oxygen species (ROS) on PS activation. We have developed a next generation PDT chlorin compound called INV043 and have shown that it has ~600 times greater phototoxicity than Talaporfin sodium, a widely used FDA approved, photosensitiser. We utilised INV043 to treat prostate cancer patients in a phase II clinical trial where patients underwent 12 cycles of PDT over 9 weeks. During each cycle, INV043 was given sublingually followed by exposure to trans-rectal laser therapy for 25 minutes at 4-hour intervals over 16 hours. We observed during the trial that INV043 was safe and well tolerated, with normal blood pressure, arterial pulse and oxygen levels observed during the therapy. Three months post treatment revealed ~44% of patients showed a positive response to treatment when evaluated by PSMA PET scan. This response rate combined with the minimal side effect and non-invasive administration makes our new generation chlorin based PDT particularly promising as a monotherapy, with the flexibility of further rounds of treatment or combination with other therapies.

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Characterization of the possible acclimation strategies and remodeling in photosystems under short-term and long-term hypersaline conditions in *Dunaliella salina*; a halophilic microalgae

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Publish consent withheld

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Functionalized phthalocyanines: synthetic challenges for improved PDT outcomes

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Functionalized phthalocyanines: synthetic challenges for improved PDT outcomes

Phthalocyanines are good candidates as photosensitisers for photodynamic therapy because the macrocyclic core absorbs are far-red wavelengths. However, it is important to functionalise them to have a chance to better improve their suitability for PDT, for example by shifting up their absorption to near infrared wavelengths, or combining them to nanoparticles and attaching them to polymers.

However functionalization of phthalocyanines is often a synthetic challenge that will be presented.

"Phthalocyanines and porphyrazines" by Ümit İşci, Fabienne Dumoulin in Fundamentals of Porphyrin Chemistry: A 21st Century Approach - Eds M. O. Senge and P. Brothers. July 2022 - ISBN: 978-1-119-12928-8 doi.org/10.1002/9781119129301.ch5

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HUMAN WAVELENGTH DISCRIMINATION THRESHOLD AND SPAN ON THE VISIBLE SPECTRUM.

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In humans, our perception of color is closely linked to the wavelength of light. Within the visible spectrum, red is associated with longer wavelengths, green falls in an intermediate range, and blue and violet correspond to shorter wavelengths.

The human eye can perceive this electromagnetic radiation within a wavelength range of approximately 380 to 780 nanometers. Individuals with normal color vision can distinguish around 150 different intervals (or colors) within this range. Color is determined intrinsically by the wavelength of monochromatic light, and our perception of color involves three main factors: hue, saturation, and brightness. Notably, our ability to differentiate between various hues (or wavelengths) is more accurate in the middle range of the visible spectrum compared to its boundaries.

From a scientific perspective, detecting these subtle differences in wavelengths within a color is highly interesting and challenging. Whit that in mind we developed a novel instrument to measure this wavelength discrimination threshold. This instrument utilizes a Tungsten-Halogen lamp as source of the visible spectrum. This light is then collected and split in two independent arms. In each arm the presence of independent automatized monochromators allows for a precise control of the transmitted wavelengths. A test software is used for running the system and an automated experiment. This is done by showing the two independent light outcomes from the monochromators that can be manipulated to be equal or distinct, allowing for an accurate discrimination at the nanometer level. This allows us for measuring the wavelength discrimination threshold across the entire spectrum, and then establish a function that describes an individual's color discrimination threshold.

Several studies where then proposed and conducted to access several types of color deficiency and age-related changes in this threshold.

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Imbalanced Expression of Chlorophyll Biosynthetic Genes Causes Photobleaching in *Arabidopsis*

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As photoautotrophs, plants use chlorophyll to harvest light energy. Thus, high levels of chlorophyll might be beneficial to plant growth. However, abnormal accumulation of chlorophyll precursors beyond the capacity of the POR enzyme could cause photobleaching. To prevent photobleaching due to the abnormal accumulation of chlorophyll precursors, the biosynthesis of chlorophyll precursors must be carefully regulated. To investigate the underlying mechanisms that fine-tune chlorophyll biosynthesis, we looked for mutants that abnormally accumulate photosensitizing chlorophyll precursors. We identified a mutant

that undergoes photobleaching due to the imbalanced expression of chlorophyll biosynthetic genes. We are currently investigating which gene is the causal gene for photobleaching.

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Induction of skin cancer by long-term blue light irradiation

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Introduction: Presently, people are not only exposed to sunlight but also to a large amount of blue light from personal computers and smartphones. This blue light has various effects on the living body. However, its effect on the induction of skin cancer is unknown. In this study, we investigated the induction of skin cancer by long-term blue light irradiation. **Methods:** Hairless mice were irradiated with blue light (LED; peak emission 479 nm) every day for one year, and a control was irradiated with white light (LED), green light (LED; peak emission 538 nm), and red light (LED; peak emission 629 nm) for one year, respectively. **Results and Discussion:** Skin cancer was induced only in the mice exposed to blue light. Long-term blue light irradiation also increased the migration of neutrophils and macrophages involved in carcinogenesis in the skin. In neutrophils, an increased expression of citrullinated histone H3 and protein arginine deiminase 4 was observed, suggesting the possibility of neutrophil extracellular trap-associated cell death (NETosis).Conversely, in macrophages, inflammatory macrophages (type 1 macrophages) increased and anti-inflammatory macrophages (type 2 macrophages) decreased due to continuous blue light irradiation. **Conclusion:** These findings suggest that long-term continuous irradiation with blue light induces neutrophil NETosis and an increase in type 1 macrophages, resulting in skin cancer (1). **Acknowledgement:** This study was supported by JSPS KAKENHI. **Conflicts of interest:** There are no conflicts of interest to declare.

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Persistent phytophotodermatitis masquerading as pyoderma gangrenosum

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Phytophotodermatitis occurs when furanocoumarin (psoralen)-containing plants contact skin and there is ultraviolet A radiation exposure. Persistent phytophotodermatitis in the absence of repeated plant exposure is not well documented.

We present a 19-year-old female who brushed past giant hogweed (Heracleum mantegazzianum) with her left extensor wrist in August 2020. Redness and discomfort developed at the site within a few hours. The next day it became vesiculo-bullous, followed by ulceration and resolution with scarring weeks later. She developed recurrence with worsening eruptions in the two subsequent summers, but had a less severe episode in 2023. On presentation to Dermatology, a necrotic ulcer suggestive of pyoderma gangrenosum was noted. Diagnostic biopsy showed inflammation with prominent deep lymphoplasmacytic infiltrate and lichenoid features. UVA provocation testing at a cumulative dose of $10Jcm^2$ to the affected site over two consecutive days produced an oedematous erythemal response, whilst a single provocation test at $10Jcm^2$ to the right wrist over one day was negative. Monochromator testing, lupus serology, porphyrins and Borrelia burgdoferi serology were negative. Patch and photopatch testing indicated a non-relevant contact allergy to limonene. Management has included topical corticosteroids, topical tacrolimus, dressings and photoprotection.

The UVA photoprovocation result supported the diagnosis of persistent phytophotodermatitis without repeated plant exposure. We are aware of two other similar cases.[1] Our case adds to the literature and is the second case to show positive UVA provocation testing. The mechanism is unclear. We postulate that there could be a cellular chemical alteration by the initial psoralen-UVA effect, resulting in localised persistent light sensitivity or, less likely, a depot of cutaneous psoralen. We wish to highlight this rare entity, which in this case mimicked pyoderma gangrenosum.

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Photosensitivity Diseases in a Paediatric Population: Lessons Learned from the Scottish Photodiagnostic Service

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Publish consent withheld

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Phytochromes inhibit the longitudinal expansion of cotyledon pavement cells

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The expansion of cotyledon pavement cells is regulated by light. However, it is not known how light regulates the expansion of cotyledon pavement cells. Here, we show phytochromes inhibit the longitudinal expansion of pavement cells in response to red light, whereas PHYTOCHROME INTERACTING FACTORs (PIFs) promote the longitudinal expansion of pavement cells. Moreover, supplementation of auxin analogue partially induced longitudinal expansion of *pifQ* pavement cell. This suggests that phytochromes inhibit the longitudinal expansion of pavement cell by modulating auxin signaling. Moreover, we found that the mutation in *LONGIFOLIA1/2* (*LNG1/2*) reduces cell longitudinal expansion in the dark, while its expression is both induced by PIF and auxin. Together we revealed that phytochromes inhibit the longitudinal expansion of pavement cells by repressing the expression of *LNG1/2* through PIF and auxin signaling.

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Pyrene-modified cyclic peptides for detecting ions in water

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The development of methods to detect metal ions in water for maintaining water quality and diagnosing metal ion-related diseases is important for improving our quality of life. In this study, we report that we succeeded in selectively detecting metal ions in water, particularly Cu^{2+} ions, using fluorescent peptides. First, we prepared seven peptides **C1**–**C7** with different sequences, each containing two pyrenylalanine units (amino acid with fluorescent dye pyrene in the side chain; Pyr) and 0, 1, or 2 His units in a 6-mer cyclic peptide by an Fmoc-based solid-phase peptide synthesis. These cyclic peptides also included a tail consisting of four Arg units to improve water solubility. The cyclic peptide **C1** (cyc-Pyr-Gly-Gly-Pyr-Gly-Glu(CH_2-CH_2-CO-Arg_4)-), which does not contain a His unit, did not show any fluorescence change when aqueous solution containing 17 metal ions were added. However, **C2**–**C7** containing one or two His units showed a fluorescence change accompanied by quenching only when an aqueous solution containing Cu^{2+} was added. On the other hand, the linear version of the **C1**–**C7** peptide showed a fluorescence response with quenching to multiple metal ions, indicating no selectivity for specific metal ions. The degree of quenching of **C2**–**C7** caused by Cu^{2+} depended on the sequence of the cyclic peptide and the number of His units. For example, **C2** (cyc-Pyr-His-Gly-Pyr-Gly-Glu(CH_2-CH_2-CO-Arg_4)-) showed poor fluorescence response to Cu^{2+}, while **C7** (cyc-Pyr-Gly-His-Pyr-His-Glu(CH_2-CH_2-CO-Arg_4)-) showed excellent fluorescence response to Cu^{2+}. Furthermore, it was revealed that **C7** was bound to Cu^{2+} in a 1:1 ratio through fluorescence titration curves and ESI-Mass spectra. These results indicate that the conformational restriction, the sequence of the peptide and the presence of His units influence the selective fluorescence detection of Cu^{2+}, and demonstrate that peptides with appropriate structures can achieve the specific fluorescence detection of Cu^{2+}.

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Recovery of the absorption band of B800 bacteriochlorophyll *a* in B800-free LH2 under neutral pH conditions

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Purple photosynthetic bacteria have LH2 as a light-harvesting protein. This protein possesses two types of bacteriochlorophyll (BChl) *a*, termed B800 and B850, which exhibit Q_y absorption bands at around 800 and 850 nm, respectively. Light energy captured by B800 BChl *a* is efficiently transferred to B850 BChl *a* in LH2. BChl *a* in the LH2 protein play crucial roles not only in photosynthetic functions but also in folding and maintaining the protein structure. However, little information is available about effects of BChl *a* on the structural property of the LH2 protein.

In this study, we analyzed an LH2 variant denoted as B800-free LH2, in which B800 BChl *a* was removed from native LH2, in order to clarify the effect of B800 BChl *a* on the LH2 structure and spectral feature. B800-free LH2 exhibited unique spectral change under neutral pH conditions, in which the Q_y absorption band of B800 BChl *a* was automatically recovered. This spectral change accompanied a decrease of the Q_y absorption band at 850 nm and tended to stop when the absorbance of B800/B850 ratio was close to that of native LH2. B800-recovered LH2 was purified and its structural and spectroscopic features were characterized. As a result, the features of B800-recovered LH2 was quite similar to those of native LH2. These results indicate that BChl *a* released by decomposition of B800-free LH2 inserts into the B800 cavity of survived B800-free LH2. This study will be helpful for understanding the structural role of B800 BChl *a* in LH2 protein.

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Simultaneous Detection of Glutathione and Iron Ions in the Frozen Tissues Using Thin-layer Chromatography and Raman Spectroscopy

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In recent years, mass spectrometric imaging (MSI) is frequently used to detect the biological molecules in the frozen tissue slice. However, it is difficult to detect the reactive metabolites such as GSH containing thiol group and small molecules with low mass. In addition, MSI needs the high-end instruments and takes a large amount of time to get molecular information. In this study, we developed a new system using thin-layer chromatography and Raman spectroscopy to detect GSH and Fe^{2+} in the frozen tissue slices easily.

First, we designed Raman probes for GSH and Fe^{2+} respectively. The Raman probe for GSH (MAL-EB) has maleimide as the reaction site for GSH and ethynylbenzene as the Raman tag. MAL-EB gives a highly polar GSH adduct upon the Michael reaction between maleimide moiety and thiol group of GSH. Therefore, after the separation of unreacted MAL-EB using low-polarity solvent, the Raman signals derived from ethynylbenzene (2110 cm^{-1}) are observed in situ. On the other hand, the Raman probe for Fe^{2+} (Phen) forms an iron complex by chelating the Fe^{2+} ion. The complex shows absorption in the visible light region around 511 nm and Raman scattering signals (1455 cm^{-1}) enhanced by resonance Raman effect. Thus, it is possible to detect the targets in the frozen tissue slice by monitoring each Raman signals simultaneously.

In fact, we applied this TLC tracing system to the mouse liver tissue. As a result, we found that it is possible to detect glutathione and Fe^{2+} in mouse frozen liver tissue with this system by monitoring the signals at 1455 cm^{-1} and 2110 cm^{-1}.

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Synthesis of DNA oligomers with Ru complex and regulation of photochemical $^1O_2$ generation by conformational change

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DNA oligomers are attracting attention as functional molecules driven in cells due to their high biocompatibility. In this study, we attempted to construct a drug that expresses its drug effect under photoirradiation conditions only in the presence of a target gene by changing the interaction between quencher and photosensitizer using structural change.

In the system, we designed a 20-mers hairpin DNA (H-ODN) and introduced a photosensitizer (ruthenium complex: Ru), and a quencher (cyclooctatetraene: COT), at both ends of H-ODN, which usually forms a hairpin-type structure. When H-ODN forms hairpin-type structure, the efficiency of singlet oxygen production is low, because of rapid energy transfer from Ru to COT. On the other hand, when target RNA complementary to H-ODN is present in the cells, H-ODN changes to a double-stranded structure. Thus, Ru and COT are separated from each other and singlet oxygen is generated upon photoirradiation. In this study, we selected a tumor-associated microRNA: miR-21 as an intracellular target and characterized the behavior of H-ODNs.

After the synthesis of H-ODNs using phosphoramidite method, we examined whether the structural change of H-ODN changes the excited state of Ru generated by photoirradiation. When H-ODN was excited in the absence of target strand, weak phosphorescence of Ru around 600 nm was observed. On the other hand, addition of the complementary strand resulted in an enhancement of the emission. We also measured the phosphorescence emission of $^1O_2$ and found that $^1O_2$ generation from H-ODN in double-stranded structure was more efficient than that from hairpin-type H-ODN. The quantum yield for generation of $^1O_2$ from double-stranded H-ODN and hairpin-type H-ODN were 0.11 and 0.02, respectively. Thus, generation of $^1O_2$ was regulated by the conformational change of H-ODN.

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Unraveling UVA1-induced Photo Modifications of Eumelanin and Pheomelanin: Insights into Pigment Darkening in Human Skin

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Solar light exposure induces pigmentary responses on the skin. UVA elicits immediate pigment darkening and persistent pigment darkening. These processes are thought to result from oxidation and/or polymerization of existing melanin and/or melanogenic precursors.

Melanocytes produce two types of pigment, eumelanin and pheomelanin. Eumelanin consists of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA), while pheomelanin consists of benzothiazine and benzothiazole units. Melanins can be analyzed through specific degradation products by HPLC. Alkaline hydrogen peroxide oxidation (AHPO) of eumelanin gives pyrrole-2,3,5-tricarboxylic acid (PTCA) and pyrrole-2,3-dicarboxylic acid (PDCA) as specific degradation products of DHICA and DHI moieties. Benzothiazole pheomelanin can be analyzed as thiazole-2,4,5-tricarboxylic acid (TTCA). Benzothiazine pheomelanin can be analyzed by reductive hydrolysis, as 4-amino-3-hydroxyphenylalanine (4-AHP) and 3-amino-4-hydroxyphenylalanine (3-AHP).

Both eumelanin and pheomelanin undergo modifications of their structures upon UVA exposure. Eumelanin exposed to UVA undergoes oxidative cleavage of the indolequinone moiety to free pyrrole-2,3,5-tricarboxylic acid (free PTCA) and cross-linking to form pyrrole-2,3,4,5-tetracarboxylic acid (PTeCA) upon AHPO. UVA exposure of pheomelanin induces oxidative conversion of the benzothiazine moiety to the benzothiazole moiety, as indicated by an increase in the TTCA/4-AHP ratio. Nevertheless, these structural modifications have never been characterized in human skin.

In this study we exposed *ex vivo* skin to increasing UVA1 doses (60, 90 and 120 J/cm^2) and characterized the induced pigment darkening before, immediately and two hours after exposure through colorimetry and HPLC. The results showed changes in the CIELAB colorimetric parameters, namely decrease in Luminance L*, yellow-blue component b* and Individual Typology Angle in UVA1-exposed samples, indicative of skin darkening. In parallel UVA1 exposure induced modifications of the levels of PTCA, TTCA, 4-AHP, and ratios of various markers, such as PTeCA/PTCA, free/total PTCA, and TTCA/4-AHP, indicative of photooxidation/degradation of melanins. Our study shows first-time evidence of UVA-induced modifications of melanins associated with pigment darkening in human skin.

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ANALYSIS OF THE MODIFICATION OF SKIN AND GUT MICROBIOTA IN PSORIASIS PATIENTS TREATED WITH PHOTOTHERAPY

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Psoriasis may be influenced by the gut microbiota due to its impact on the regulation of systemic immunity and phototherapy may modify the skin microbiota through its antimicrobial activity. The aims were:to relate intestinal and cutaneous bacterial and fungal abundance in patients with psoriasis,and to determine the impact of UVB-NB.

M&M:Patients with psoriasis provided faecal samples and samples of healthy and affected skin collected with a swab before and after treatment with phototherapy. Bacterial and fungal composition was determined by mass sequencing on the MiSeq platform (Illumina). Taxonomic assignment was performed with the databases Silva 138 and UNITE. In addition,colourimetry, blood pressure,quality of life and psoriasis severity scales and analyses with IL-6,lipid profile,vitamin B12 and folic acid, were performed before and after phototherapy.

RESULTS:8 patients were included, mean age of 48.8[30-60],5 women and 3 men, 75% with phototype 3 and 25% with phototype 2. Statistically significant differences were found in PASI,BSA and IGA scales(p=0.01).In colourimetry, there was a significant increase in erythema.There was a statistically significant reduction in IL6 levels.A positive correlation was detected between increased erythema and improved PASI(0.762, p=0.02).Alpha diversity values were significantly higher in healthy skin, with a marked reduction in the number of species in affected skin. No relevant results were observed in faeces. In healthy skin,Malasseziaceae,Cladosporiaceae and Herpotrichiellaceae were the most abundant fungal families, whereas taxa with no clear taxonomic assignment were predominant in affected skin. In terms of bacteria, the beta diversity of psoriasis-affected skin after phototherapy was very similar to that of healthy skin before phototherapy.

CONCLUSION:Swab sampling is valid for studying microbiota by mass sequencing.Many of the fungal readings in psoriasis-affected skin could not be taxonomically assigned,indicating the presence of fungal species that have not yet been included in databases.Treatment with phototherapy has an antimicrobial impact on both affected and healthy skin.

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Identification and characterisation of phyllochlorin sodium, a novel chlorin-derived photosensitiser

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Photodynamic therapy (PDT) is a minimally invasive therapeutic approach for the treatment of various conditions including cancers. A key feature of PDT is the light-absorbing photosensitiser, which is activated in the presence of oxygen and a specific wavelength of light, leading to the generation of reactive oxygen species (ROS) and cell death. 'First generation' photosensitisers have been commercialised to treat numerous malignancies including lung and skin cancers. However, disadvantages such as low purity, poor stability, photosensitivity, and poor absorbance have prompted the development of novel photosensitisers to improve PDT outcomes.

We have identified a new chlorin-based photosensitiser – Phyllochlorin Sodium. Phyllochlorin sodium with improved characteristics of chlorin-based photosensitisers.

Our study has demonstrated that phyllochlorin sodium can generate a high yield of ROS in the form of singlet oxygen, with rapid cellular uptake and localisation to subcellular regions in the endoplasmic reticulum. When compared to similar photosensitisers such as chlorin e4 disodium salt, chlorin e6 trisodium salt (Photolon), chlorin e6 dimeglumine salt (Photodithiazine) and chlorin e6 trimeglumine salt (KIA), phyllochlorin sodium had significantly greater phototoxicity upon light activation, with low toxicity in the absence of light. In tumour-bearing mice, phyllochlorin sodium was found to accumulate and remain in tumours when compared to normal cells.

We present here our next generation phyllochlorin sodium photosensitiser and demonstrate its increased stability and enhanced phototoxicity in cancer cells compared to similar compounds. Our study offers new insight into the development of a novel family of photosensitisers and their potential clinical efficacy and application for PDT.

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Synthesis of new derivatives of purpurine imide for potential phototherapy dynamic applications

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Chlorins are among the most interesting photosensitizers for photodynamic therapy because they exhibit high quantum yield of the singlet oxygen generation and absorbance in the near infrared. Indeed the penetration depth of light into skin increases with wavelength from the UV to the near-infrared light range. Even if chlorins can be synthesized, they are present in the nature and

the most abundant natural chlorin is the chlorophyll. After aceton extraction of chlorophyll a from *spirulana maxima*, it is possible to synthesize a derivative, the purpurin 18, which has an absorbance around 700 nm thanks to an additional anhydride exocyclic ring compared to the chlorin p6 whose maximum absorbance wavelength is at 650 nm.[1] The disadvantage is that this anhydride exocyclic ring is very reactive and can be easily opened by a nucleophile to lead to a derivative of chlorin p6. To preserve a cycle and thus the absorbance around 700 nm, previous work have shown that it is possible to open purpurin 18 with an amine and to recyclize to lead to purpurin imide derivatives which have also shown very important phototoxicity.[2] In this work we have therefore synthesized differents derivatives of purpurine imide, which have different reactive functional groups such as amino, sulfhydryl, maleimide, azide and alkyne to allow subsequent functionalization and open up the possibilities of using them. The structures of all new derivatives of purpurine imide were characterized by NMR and UV/visible spectroscopy and mass spectrometry.

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Antibacterial activity of photoactivatable CO-releasing molecules (photoCORMs) and corresponding materials based on rhenium(I) complexes and cellulose nanocrystals

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Antibiotic-resistant bacteria represent a growing threat to global health, **and there is a urgent need to address this issue.** Carbon monoxide (CO) has been identified as an inhibitor of bacteria proliferation and a potent antibacterial agent. Its controlled delivery by photoactivatable CO-releasing molecules (photoCORMs) could be an attractive alternative to conventional antibiotics [1], especially if these molecules are incorporated into nanomaterials with the aim to increase their biocompatibility.

In the present work, a tricarbonylrhenium(I) complex (**Re-Phe(TPP)**) and a hydrophobic analogue substituted by an adamantyl moiety (**Re-Ada(TPP)**) were developed. These complexes were integrated to a biocompatible cellulose nanocrystal (CNC) matrix [2]. When irradiated in the near UV, the free photoCORMs generate rapidly one molecule of CO and small amounts of singlet oxygen ($^1O_2$). Their decarbonylated photoproducts generate only $^1O_2$, on a prolonged period of time. In the CNC material, the diffusive species (CO and $^1O_2$) were the most active ones. None of these systems showed bactericidal activity against *Pseudomonas aeruginosa*. In contrast, **Re-Ada(TPP**) showed clear photochemical activity against *Staphylococcus aureus*, while **Re-Phe(TPP)** was a very good antibacterial agent in the dark. The decarbonylated photoproduct **D-Re-Phe(TPP)** showed moderate activity, suggesting that part of the efficiency is linked to the production of CO. The photoCORMs adsorbed on CNCs were rather ineffective. This suggests that the direct biological action of photoCORM and/or the generation of CO and $^1O_2$ near their biological target are necessary for good antibacterial activity [3]. This work allows to identify the limits of Re(I) photoCORMs and corresponding nanomaterials, and it provides good indications on how to improve their design.

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Chlorin e6 is not a U.S. FDA Approved Photosensitiser

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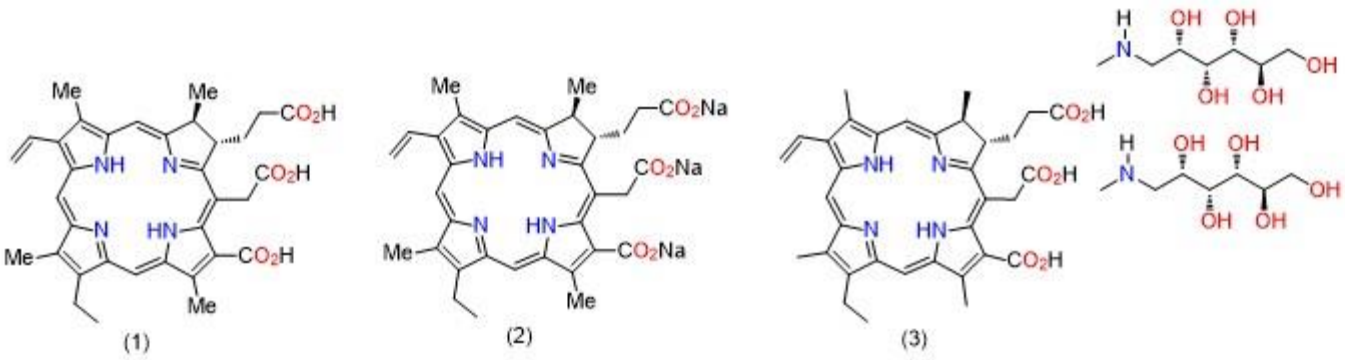
Chlorin e6 (1) also known as Phytochlorin e6 has over 3,200 literature references. Recently a number of publications have indicated that Chlorin e6 is a U.S. FDA approved photosensitiser. While Chlorin e6 appears on the FDA database, and has a Unique Ingredient Identifier (UNII), it is not an FDA approved photosensitiser.



Chlorin e6 itself has low solubility in aqueous systems and is generally used as one of its more water-soluble salt forms (2), (3) and (4). The chlorin e6 trisodium salt (2) administered with polyvinyl pyrrolidone (PVP) is known as Photolon is approved in the Russian Federation and the Republic of Belarus for the treatment of a number of cancers. Photodithiazine (3) is the dimeglumine salt of Chlorin e6, also approved in the Russian Federation. The trimeglumine salt (4) known as KAE is currently under evaluation in China as an alternative photosensitiser for cancer indications. All three salts are considered 2nd generation photodynamic agents.

Although these salts have provided valuable learnings, and are no doubt active as photodynamic agents, they are to our knowledge not approved, at this stage, in western countries as photosensitisers.

We have found that all three salts of chlorin e6 are not stable in solution, producing the same major degradation product which we have identified. This degradation product has been tested using in-vitro anticancer screens and has been found to be a more powerful photosensitiser than any of the three chlorin e6 salts. A patent application has been filed (RMWC Limited) covering this invention for application in cancer treatments.

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Photoreception and signaling in bacterial phytochrome revealed by single particle cryo-EM

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Phytochromes are red-light photoreceptors discovered in plants with homologs in bacteria and fungi that regulate a variety of physiological responses. They display a reversible photocycle between two distinct states: a red-light absorbing Pr and a far-red light absorbing Pfr state. The photoconversion regulates the activity of an enzymatic domain, usually a histidine kinase (HK). The molecular mechanism that explains how light controls the HK activity is not understood because structures of unmodified bacterial phytochromes with HK activity are missing. Here, we report three cryo-EM structures of a wild-type bacterial phytochrome with HK activity determined as Pr and Pfr homodimers and as a Pr/Pfr heterodimer with individual subunits in distinct states. We propose that the Pr/Pfr heterodimer is a physiologically relevant signal transduction intermediate. Our results offer insight into the molecular mechanism that controls the enzymatic activity of the HK as part of a bacterial two-component system that perceives and transduces light signals.

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The German Solar UV Monitoring Network – Implementation of diode array radiometers and low cost broadband filter radiometer

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The German Federal Office for Radiation Protection (BfS) operates a nationwide network for solar ultraviolet (UV) radiation monitoring in cooperation with the Federal Environment Agency, Germany's National Meteorological Service and other associated institutions.

Until 2017, scanning double monochromators (DM) were used within the network for spectral-resolved measurements to ensure our requirements on accuracy. However, these devices are expensive and high-maintenance. In addition, the measurement time of several minutes is a disadvantage in case of fast changing cloud conditions. An alternative system was found with a diode array radiometer using BTS technology (BTS). Comparative validation measurements of BTS and DM systems have shown that the more cost-effective BTS systems achieve sufficient stray light reduction (dynamic range) with a shorter measurement time than DM and high spectral resolution. This allows the spectral UV irradiance to be determined more accurately at fast changing

cloud conditions. For this reason, further stations in the UV monitoring network were expanded with BTS diode array radiometers. One of them was installed in the high mountain region Alps, where often fast changing cloud conditions as well as the highest solar UV irradiance in Germany appear.

To inform the public about the current solar UV irradiance the BfS publishes daily courses of the UV Index as derived from the measurements of all measurement stations continuously updated over the day. However, the information is generally valid to the region of the measurement station due to the strong dependence of the solar UV irradiance on the cloudy conditions. For comprehensive information, the number of measurement stations of the German solar UV monitoring network is insufficient. To this end, the existing network is being expanded to further twenty additional stations equipped with small and low-cost UV Index sensors which achieve the desired level of accuracy for UV Index determination. The expansion is made in cooperation with the German ODL (ambient gamma dose rate) network with its 1800 stations.

In 2024 the network includes 14 stations for spectrally resolved measurements and more than 30 additional stations equipped with new broadband filter radiometer for direct measurement of the erythemal irradiance. The expansion of network with a special focus on the applied devices, the validation and the measurement results as well as the communication of the current erythemal irradiance to the public will be presented.

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Increasing Solar UV Radiation in Dortmund, Germany, and Uccle, Belgium – Results of Long-Term UV Monitoring

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Increasing solar ultraviolet radiation (UVR) has the potential to adversely affect humans, life on Earth and the environment, impacting both human health and the sustainability of ecosystem. Precise measurements over several decades and thorough data analyses are crucial for identifying and understanding changes in ground-level solar UVR.

For the locations Dortmund in Germany and Uccle in Belgium, we processed and analysed spectrally resolved data on solar UVR from UV monitoring stations. Influencing factors such as total column ozone, global radiation and sunshine duration were considered in analysis and discussion. The differences in annual UV dose between the two locations are consistent with the variations in global radiation. A detailed analysis of ozone influence in Dortmund reveals the impact of the normal ozone annual course and low-ozone events on the UV data separately.

An advanced linear model was developed and applied for the trend analysis. The results show a statistically significant increase in the monthly mean standard erythemal dose and UV Index values from 1997 to 2022 in both Dortmund (SED, 4.9% per decade, UVI, 3.2% per decade) and Uccle (SED: 7.5% per decade, UVI: 5.8% per decade). In Dortmund, the global radiation increases equally to the SED and UVI data. Sunshine duration, which primarily depends on the change in cloud cover, increases by 11.3 % per decade, roughly twice as much as global radiation. Total column ozone shows a slight but statistically significant decrease in summer months (0.9% per decade).

The overall results suggest that the changes in monthly UVI and SED mean values are primarily driven by changes in global radiation, which in turn are primarily caused by changes of sunshine duration. A decreasing summer total column ozone may also influence UVI and SED changes; but on a minor level.

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Digital monitoring of personal solar dose

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Longitudinal multi-subject photobiological studies that require accurate monitoring of the participants' solar exposure are currently challenging and limited. Dosimeters or wearable devices are expensive to acquire and maintain and compliance among users can be low.

Here an innovative digital solution that provides personal solar dosimetry data using only a smartphone (no light sensors or dosimeters) has been investigated. The smartphone app (ExpoDose®, siHealth Ltd) is used to track whole-body exposure to spectral solar radiation. It's based on a patented technology already validated scientifically [1] combining real-time satellite data, radiation transfer modelling and AI-enabled automatic assessment of indoor/outdoor position. The app seamlessly tracks the

solar exposure of multiple users for any number of action spectra (e.g., erythema, vitamin D synthesis, UVA) and body sites (e.g. scalp, face), providing the collected data to study investigators via a web-portal.

The results of a 6-month study into the accuracy and practical use of the app will be presented. Solar erythemal irradiance (global horizontal) data collected by the app have been compared to ground station measurements and found to have an R^2 correlation coefficient of 0.90 and a mean absolute error of 21%. The automatic indoor/outdoor detection component has been tested and its detection accuracy ranged from 84% (iOS) to 92% (Android). This demonstrates the accuracy of the app, which, when coupled with its convenience, makes it a tool that could significantly enrichen and diversify the possibilities for epidemiological and photobiological studies.

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Intratumoral therapeutic cancer vaccination and cure of mice after photochemical internalisation (PCI) in combination with a HPV-peptide and the TLR3-agonist poly(I:C)

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In the development of peptide- or protein-based therapeutic cancer vaccines, suboptimal priming of antigen-specific CD8+ cytotoxic T lymphocytes (CTL) due to insufficient MHC class I presentation remains a major problem. Adjuvants are therefore important to improve cross-presentation on MHC class I and to prime CTLs. In this study, we implemented the endosomal escape method photochemical internalisation (PCI) in order to enhance the cytosolic delivery of a human papilloma virus long (HPV-L) vaccination peptide in immunocompetent mice in combination with the Toll-like receptor 3 (TLR3) agonist Poly(I:C) as adjuvant (double stranded RNA mimicking virus infections). In C57BL/6 mice with established dermal syngeneic tumours of the HPV TC-1 cancer model, PCI-mediated vaccination with a synthetic HPV-L derived from HPV16 in combination with poly(I:C) resulted in significant activation of antigen-specific CD8+ T-cells and caused anti-tumour effects. In comparison to PCI alone, combining intratumoural PCI, HPV-L and poly(I:C) resulted in complete responses and cure of all mice (8/8) treated as compared to mice treated with either HPV-L and poly(I:C) (0/8) or PCI and HPV-L (0/8). In conclusion, PCI strongly improved immunity of TLR3-agonist adjuvanted polypeptide antigens. The PCI-based vaccination strategy has a promising potential in the development of therapeutic cancer vaccines and warrant further development towards clinical testing.

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Photoimmunotherapy â how light collaborates with interferon to induce anti-tumor immune responses

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