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The Nobel Lecture

GFP: Unexpected Consequences From a Serendipitous Discovery

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Fortieth anniversary of the European Society of Photobiology. History and significance in the scientific community

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The European Society for Photobiology (ESP) was established in 1985 with the mission to coordinate and promote all aspects of photobiology across Europe. Its aim is to communicate and optimize achievements in scientific, technological, and medical arenas related to photobiology. ESP organizes a large variety of activities of importance for researchers, clinicians and others with engagements in photobiological topics, including biannual congresses, education and the development of young scientists such as organizing a photobiology school and also offers fellowships and awards.

The history and significance of ESP in the scientific community will be presented.

Engineering Carbon-Based Phototransducers for Cell Stimulation and Vision Restoration

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Life-machine interfacing holds great potential in regenerative medicine, robotics, and assistive technologies. In this talk, I present intra-membrane molecular transducers as a novel, non-genetic strategy for cell opto-stimulation. These phototransducers, based on amphiphilic photochromic molecules, spontaneously insert into the plasma membrane and convert light into electrical signals, directly modulating cellular activity. By chemical design, we engineered variants that selectively influence membrane capacitance, conductance, and surface charge. Experimental data from different cell types will be shown, along with a demonstration of restored light sensitivity in blind retinas. These findings offer a new route for developing photo-responsive systems through non-covalent membrane targeting, opening new perspectives for seamless life-machine integration[1,2].

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Light-triggered Metallodrugs for Phototherapy: from Concept to Clinical Studies

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Keywords: photodynamic therapy; TLD1433; ruthenium; excited states

Photodynamic therapy (PDT) is a special branch of photomedicine that employs a photosensitizer, light, and oxygen to destroy cancer cells with spatiotemporal selectivity. The photosensitizers used for PDT have historically been organic molecules, specifically porphyrins and other tetrapyrrole-related structures. Given the important role of metals in medicine, metallated analogs of these traditional systems (as well as metal complexes of markedly different architectures) have attracted considerable attention in recent years. TLD1433, a metal complex designed in our laboratory, is an example that is also the first ruthenium-based photosensitizer for PDT to advance to clinical trials. This presentation will provide an update on translational activities and outcomes and discuss some of our current research efforts that were shaped by the design and development of TLD1433 as a bladder cancer PDT agent.

Photosynthesis in the Far-Red: From Cyanobacteria to Plants

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Photosynthesis is the biological process by which light energy is converted into chemical energy, sustaining nearly all life on Earth. In oxygenic organisms, it typically operates within the photosynthetically active radiation (PAR) range of 400–700 nm, where chlorophyll a absorbs most efficiently. However, some cyanobacteria have evolved the ability to perform photosynthesis beyond this range, using far-red light. Studying these systems provides valuable insight into how the photosynthetic machinery can adapt to capture and utilize lower-energy photons.

The main challenges in far-red photosynthesis lie in both the efficient harvesting of long-wavelength photons and their effective use in photochemical reactions, given their reduced energy. To overcome these limitations, photosynthetic organisms employ a variety of strategies: they introduce new far-red–absorbing pigments and tune the absorption properties of existing pigments through their protein environment. These adjustments are accompanied by structural adaptations in antenna complexes and photosystems that help maintain high photochemical efficiency.

In this presentation, I will discuss the natural strategies that support far-red photosynthesis and how these solutions may guide efforts to engineer similar capabilities into plants. This work opens promising avenues for expanding the spectral range of photosynthesis and improving light-use efficiency in future crop systems.

Light Sensing in Marine Diatoms: from molecular mechanisms to ecosystems

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Keywords: Marine Photobiology, Diatom, Phytochrome, Circadian Clock

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.

One of our major research interests is understanding the biology of diatoms—a dominant class of phytoplankton that stand at the crossroads of several evolutionary lineages [1]. Diatoms represent the most species-rich group of microalgae and are distributed worldwide, from tropical to polar ecosystems. Their diverse lifestyles and survival strategies likely stem from a remarkable ability to adapt to highly variable light environments. In recent years, functional studies in diatom model species combined with environmental investigations have led to the identification of new regulators of photosynthesis, photoreceptor variants, and a long-sought circadian clock controlling diatom responses to periodic light/dark cycles [2, 3]. 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 relevant physiological responses [4]. 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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Photodermatology – Quo Vadis?

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Keywords: Phototherapy; Dermatology; Photoprotection; Skin Disease

The lecture begins with a brief overview of the history of photodermatology, starting with the discoveries at the turn of the century that led to Nils Finsen being awarded the Nobel Prize. Further milestones followed in parallel with the development of modern light sources such as fluorescent tubes, lasers and LEDs as well as photosensitizers, which together revolutionized the treatment of inflammatory skin diseases, especially psoriasis. With the introduction of topical photodynamic therapy, a powerful tool for the treatment of superficial skin cancer became available. At the same time, clinical and experimental research led to important advances in the understanding of UV-induced skin diseases, including acute and chronic photodamage and photocarcinogenesis. The progress made in the field of photoprotection in recent decades, in particular the development of broad-spectrum sunscreens and systemic photoprotectors, is still ongoing. In recent years, however, the development of effective pharmacological therapies for psoriasis and other inflammatory skin conditions has superseded dermatologic phototherapy. This led to the reduced availability of light sources, associated expertise and to less associated research. However, as solar radiation remains the most abundant environmental factor that human skin is exposed to, and given the impact of climate change on terrestrial solar radiation, research into its beneficial and harmful effects on the skin will continue to play a central role in medicine also in the future.

Photosynthetic proteins in action! Towards a real-time investigation of how light-harvesting is regulated in photosynthetic organisms

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Plants and algae provide a natural example of how solar energy can be converted into chemical energy in the presence of oxygen while preventing photodamage. It has now been established that plants and algae prevent photooxidation by activating a rapidly inducible and reversible photoprotective mechanism at the level of their light-harvesting complexes. However, the precise activation process of this photoprotective mechanism remains unknown. I will here introduce our current understanding of how light-harvesting is regulated in plants and algae and, more generally, in oxygenic photosynthetic organisms. I will then highlight the spectroscopic and computational tools under development by our recently-established group at ICFO, aimed at unraveling the molecular mechanisms governing the activation of photoprotection in photosynthetic organisms – in real time.

Understanding both the mechanism and the rate at which plants can activate or deactivate photoprotection may provide answers to long-standing open questions in the fields of biophysics and physical chemistry. This knowledge will also be instrumental in inspiring new studies focused on maximizing plant productivity through the optimization of photoprotective responses.

Emergent Photoactivated Anticancer Therapies: A Molecular Description

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Keywords: Transition Metals; Quantum chemistry; Molecular dynamics

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 O2 mediated type I and/or type II photodynamic therapy (PDT) photoreactions. However, the physiological conditions of solid tumors often imply low levels of molecular dioxygen, limiting the outcome of the already clinically approved drugs.[1]

The present contribution will describe recent approaches to circumvent the hypoxia problem from a multidisciplinary perspective, emphasizing however the contributions of computational chemistry in the elucidation of the molecular and electronic mechanisms behind the photodamage. The photoprocesses that will be considered are mediated by molecules based on heavy transition metals[2-9] or metal-free agents.[10] The contribution will show recent results on how these non-canonical photosensitizers respond upon light absorption, and how this information reveals the photochemical mechanisms responsible for the biological damage.

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Monday, August 25th

Photodynamic therapy and immunogenic cell death in cancer immunotherapy

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Keywords: glioma, apoptosis, ferroptosis, DC vaccines

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, act as adjuvants that facilitate the recruitment and maturation of antigen-presenting cells. However, the presence of these adjuvant DAMP 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 (DCs), particularly through the generation of neo-epitopes. Many anticancer agents and strategies induce ICD, but despite their robust effects *in vitro* and *in vivo* in mice, clinical translation remains challenging.

In this presentation, I will first discuss the main principles of ICD modalities (*e.g.,* apoptosis and ferroptosis) and their role in cancer immunotherapy. Next, I will explore the molecular mechanisms through which photodynamic therapy (PDT) can induce different ICD modalities and will discuss the data on the therapeutic efficacy and underlying molecular mechanisms responsible for the generation of anti-tumor immunity by DC vaccines loaded with cancer cell lysates that have undergone ICD. In the final part, I will present data on temoporfin-mediated PDT to induce ICD. Altogether, these findings highlight the potential of ICD-inducing strategies, including PDT, to advance the development of effective cancer immunotherapies.

Acknowledgments
The CDIT Laboratory is supported by the Flanders Research Foundation (FWO) and the Belgian National Fund for Scientific Research (F.R.S.-FNRS) under the Excellence of Science (EOS) program (40007488), FWO grant (G016221N), Special Research Fund (BOF) grants from Ghent University (BOF/IOP01/O3618, BOF/IOP/2022/ 033, BOF23/GOA/029), and IOF grants from Ghent University ('PULSE' F2023/IOF-ConcepTT/033 and 'IMMUNO-FER-GUARD' F2023/IOF-ConcepTT/106).

Enhancing photodynamic therapy and anti-tumor immunity: the critical role of drug delivery

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Keywords: Photodyanmic therapy, immune checkpoint blockers, cell uptake, enhanced tumor delivery

Photodynamic therapy (PDT) combines a photosensitizer and light to generate reactive oxygen species (ROS) for targeted tumor destruction. Beyond local effects, PDT can trigger anti-tumor immunity, supporting long-term cancer control.

Redaporfin, a tetraphenyl bacteriochlorin, showed limited efficacy in the orthotopic 4T1 breast cancer model, even when combined with immune checkpoint blockers (ICBs). This model, characterized by a fibrotic stroma and an immunosuppressive tumor microenvironment, hampers drug delivery, and the large molecular size of both redaporfin (~1000 Da) and ICBs further limited their tumor accumulation [1].

To overcome this, we employed photoacoustic waves (PAWs) — 60 bar compressive waves previously shown to enhance tissue permeability [2]. In orthotopic 4T1 tumors, PAW-priming improved delivery of redaporfin and ICBs, significantly extending overall survival [1,2]. However, no full tumor eradication was achieved.

To further enhance tumor penetration, we developed a smaller derivative of redaporfin: a fluoro-diphenyl bacteriochlorin carboxamide, named LUZ51. This molecule, with a low molecular weight (595 g/mol) and high amphiphilicity, is rapidly internalized by cancer cells and, after just 30 minutes of incubation, effectively kills 4T1 cells at nanomolar concentrations (IC_{50} = 28 nM; 1 J/cm²).

In CT26 tumor-bearing mice, PDT with LUZ51 (0.15 mg/kg; 20 or 40 J/cm²) induced pronounced edema and tumor necrosis, curing ~ 60% of mice. The involvement of T cells was confirmed, as cured mice resisted subsequent rechallenge with live cancer cells, and no cures were observed in nude mice. Early post-treatment IL-6 elevation and neutrophil infiltration indicated acute inflammation.

In the 4T1 orthotopic model, biodistribution studies confirmed tumor accumulation of LUZ51, and single-cell analysis demonstrated its association with tumor cells. PDT with LUZ51 (0.15 mg/kg; 20 J/cm²) significantly extended median survival. One mouse remained tumor-free for 65 days and resisted rechallenge, indicating durable immunity. Future plans include combining PDT with LUZ51 and ICBs, using PAW to further enhance drug delivery to solid tumors.

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Enhancing CD8+ T-Cell Responses with PCI-Based Therapeutic Cancer Vaccination

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Photochemical internalisation (PCI) is a PDT-based drug delivery technology that uses light to release endocytosed macromolecules by disrupting endosomal/lysosomal membranes. PCI enhances the efficacy of cancer immunotherapies, including immunotoxins and peptide-based vaccines, which often struggle with suboptimal priming of antigen-specific CD8+ T-cells (CTLs). Normally, antigens captured by antigen-presenting cells (APCs) are degraded in lysosomes, leading to MHC class II presentation and CD4+ T cell activation, with minimal MHC class I presentation and CTL induction. The PCI method improves cytosolic release and MHC class I presentation of antigens on APCs, boosting CD8+ CTL activation. In a Phase I trial, PCI-enhanced prophylactic vaccination was safe and elicited cellular immune responses. This talk will cover novel therapeutic cancer vaccines combining PCI with tumour-specific antigens and adjuvants. Two administration methods were tested: subcutaneous and intratumoural (24 hours before light activation). Mice were immunized three times at 7-day intervals. Fimaporfin-PCI-mediated vaccination with an HPV-based peptide and the Toll-like receptor 3 (TLR3) agonist poly(I:C) activated antigen-specific CD8+ T-cells and induced strong anti-tumour responses in the murine HPV16 cancer model TC-1. Both strategies were highly effective, with direct tumour injection being safer than subcutaneous vaccination. The combination with an adjuvant was crucial to obtain complete responses. In conclusion, PCI hold significant potential for advancing therapeutic cancer vaccines, offering a promising way for more effective and safer cancer care.

PDT and immunotherapy of cancer
Chair: Pål Selbo

Immunological effects after photodynamic therapy in blood and glioma tissue by using orthotopic Fisher glioma rat model

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Glioblastoma is one of the most common and lethal brain tumors with a poor prognosis and a median survival of around 14 months. This study investigates blood immune cell composition in photodynamic therapy (PDT) and photochemical internalization (PCI)-treated glioma-bearing rats (from F98 glioma cells) alongside pathological characterization of the tumors. The aim was to evaluate the impact of PDT and BLM-PCI on lymphocyte subset distribution in blood and to assess if effects on blood immune cells correlate with histological findings on tumor size/progression and immune cell presence/infiltration in tumor.

For PCI treatment, rats were injected with the photosensitizer $TPCS_{2a}$ and bleomycin (BLM) followed by tumor light exposure with a 652 nm diode laser (0.6 W). Blood and brain tissue samples were collected 6 days post illumination. After sacrifice, aortic out-bleeding was performed under anesthesia and the brains were preserved in formalin (4%) for pathological examinations. To assess treatment effects on immune cell composition, blood samples were stained with a viability dye (Fixable Viability Dye eFluor 780) and fluorescent antibodies to identify lymphocytes, CD4+ T-cells, CD8+-T-cells, B-cells, and NK-cells. Flow cytometric analysis was performed on a BD LSR II flow cytometer. Sections form formalin-fixed and paraffin-embedded tumor tissue were routinely stained with hematoxylin/eosin and Iba1 antibody to assess tumor (size/localization/progression) and the distribution of immune cells /macrophages.

Flow cytometry data revealed a decrease in most lymphocyte subsets in PCI- and PDT- treated groups, with a more pronounced decrease in the PCI group. Notably, PCI differed markedly from both BLM- and PDT-group and healthy controls. Immunohistochemical analysis of macrophage distribution in brain tissue showed a trend to lower number of tumor-associated and tumor-infiltrating macrophages in the brain of PCI-treated rats, while PDT and BLM treatment did not affect the number of macrophages recruited to the tumor tissue. An overall assessment may indicate that PCI treatment reduces immune cell numbers in blood and tumor compared to PDT and BLM treatment.

Utilizing Photoimmunotherapy to Combat Multidrug Resistance

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Keywords: Photoimmunotherapy; chemo-resistance, P-glycoprotein; ovarian cancer;

Ovarian cancer is the deadliest gynecologic disease with a 5-year survival rate of ~30% for advanced stages [1,2,3]. Development of chemo-resistance is a major cause of this poor prognosis and is attributed to 90% of ovarian cancer deaths [4]. ATP-binding cassette (ABC) drug efflux transporters, particularly P-glycoprotein (P-gp, ABCB1), confer multidrug resistance and present a target for inhibition strategies [5]. Existing small molecule inhibitors of P-gp often lack cancer selectivity and are thus associated with normal tissue toxicity [5]. Our team has demonstrated that traditional photodynamic therapy (PDT), employing the FDA-approved photosensitizer benzoporphyrin derivative (BPD), can effectively inhibit ABC transporters in cancer cells through both direct structural damage and ATP depletion [6,7,8]. PDT is a localized treatment that utilizes a photosensitizer—a light-activated drug—and light to generate cytotoxic reactive oxygen species. However, BPD alone lacks cancer selectivity, making it less suitable for addressing disseminated tumors, such as those found in advanced-stage ovarian cancer. To improve cancer selectivity, we conjugated BPD to an anti-epidermal growth factor receptor (EGFR)-targeting monoclonal antibody to form a photoimmunoconjugate (PIC). PIC's ability to inhibit or evade P-gp efflux was evaluated using an ovarian cancer cell pair of a chemo-sensitive parental line and its chemo-resistant, P-gp-overexpressing subline. We demonstrate that PIC undergoes less P-gp-mediated efflux than freeform BPD. PIC-PIT retains BPD's ability to decrease P-gp function in a chemo-resistant ovarian cancer cell line, demonstrated as an increase in P-gp substrate accumulation and a decrease in P-gp expression. These findings highlight the role of PICs, and the importance of understanding their interactions with ABC transporters, in combating multidrug resistance in advanced-stage cancer.

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Enhancing BCG immunotherapy with photochemical treatment

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Intravesical immunotherapy with Bacille Calmette-Guérin (BCG) is the standard of care for high-risk non-muscle-invasive bladder cancer. While its mechanism is not fully understood, BCG significantly delays tumour recurrence. However, 25–45% of patients do not respond, and up to 40% eventually relapse.

We hypothesise that combining BCG with $TPCS_{2a}$-based photochemical treatment (PCT) enhances the efficacy and durability of the antitumour response. $TPCS_{2a}$ is an amphiphilic photosensitiser that localises to the plasma membrane and endosomes during endocytosis. Upon light activation, it generates reactive oxygen species, particularly singlet oxygen, leading to dose-dependent membrane damage. This photochemical effect, used in photodynamic therapy or photochemical internalisation, is immunogenic. Recent data show that combining $TPCS_{2a}$-based PCT with BCG enhances tuberculosis vaccine efficacy by increasing local inflammation and CD4/CD8 T-cell responses—potentially translatable to bladder cancer.

We generated stable BCG–$TPCS_{2a}$ complexes and evaluated them in vitro using MB49 bladder carcinoma cells, comparing against monotherapies (BCG or photoactivated $TPCS_{2a}$ alone). Cytotoxicity was assessed by MTS assay, transcriptomic differences by RNA sequencing, and immunogenic cell death by DAMP release. In vivo, MB49 tumours in immunocompetent mice were treated intratumourally with light-activated BCG–$TPCS_{2a}$, repeated three times at one-week intervals. Tumour growth was monitored, and tissues were collected for immunohistochemistry, flow cytometry, and splenocyte stimulation assays.

BCG–$TPCS_{2a}$ induces concentration- and light-dose-dependent cytotoxicity in vitro. RNAseq reveals distinct treatment signatures. In vivo, combination therapy delays tumour growth and induces complete remission in 20–40% of mice in the best-tested regime. Analysis of tumour and spleen tissues is ongoing.

PDT and immunotherapy of cancer
Chair: Pål Selbo

Photoimmunotherapy Modulates P-glycoprotein Function In Vivo

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Keywords: Photoimmunotherapy, chemo-resistance, P-glycoprotein, ovarian cancer

EXTENDED ABSTRACT

Chemoresistance remains a major obstacle in ovarian cancer therapy, with 80–90% of cases recurrent within 6 months exhibiting multidrug resistance [1]. A key driver of this resistance is the overexpression of P-glycoprotein (P-gp/ABCB1), an efflux transporter that reduces the intracellular accumulation of chemotherapeutic agents [2,3]. Although small molecule P-gp inhibitors have been investigated, their clinical use is limited by systemic toxicity and adverse drug interactions [4,5], highlighting the need for a safer, targeted strategy. We previously demonstrated that photodynamic therapy (PDT) using freeform photosensitizer (benzoporphyrin derivative) can modulate P-gp function in vitro [6]. To improve tumor selectivity, we developed a photoimmunoconjugate (PIC)—an antibody-photosensitizer conjugate—and, for the first time, showed that intraperitoneal photoimmunotherapy (PIT) using PIC can effectively target P-gp in a mouse model of peritoneal carcinomatosis. The PIC was filter sterilized and characterized, prior to being activated in vivo using intraperitoneal light exposure. P-gp function was evaluated by isolating tumor cells post-treatment and assessing Rhodamine 123 accumulation via flow cytometry. Compared to controls, PIC-PIT significantly increased intracellular dye retention, indicating effective inhibition of P-gp activity without changes in protein expression. This work presents the first in vivo evidence that photoimmunotherapy can selectively inhibit P-gp function, offering a promising approach to overcome chemoresistance without the systemic toxicity of conventional inhibitors. Ongoing studies aim to optimize treatment conditions and assess the durability of transporter inhibition.

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PDT and immunotherapy of cancer
Chair: Pål Selbo

Anti-tumoral effect of light-activated CD351 in breast and pancreatic cancer

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Keywords: Photodynamic Therapy, Chlorophyll Derivatives, Breast Cancer, Pancreatic Cancer, 3D models, CD351

Cancer remains one of the leading causes of death worldwide [1]. According to the World Health Organization (WHO), every 60 s, four women are diagnosed with breast cancer (BC), and one of them will die from the disease [2]. Although pancreatic cancer (PC) is less incident, the five-year survival rate is only 12% [3]. Triple-negative BC (TNBC) is particularly aggressive and has a poor prognosis due to the absence of hormone receptors, which limits treatment options [4]. On the other hand, pancreatic ductal adenocarcinoma (PDAC) is among the deadliest forms of cancer, primarily because of its lack of symptoms, high metastatic potential, and resistance to conventional therapies [5]. These challenges highlight the urgent need for safer and more effective treatment strategies to improve patient outcomes.

In this communication, we will present the potential of CD351, a natural chlorophyll-derived molecule, as a photoactive compound for photodynamic therapy (PDT) against BC and PDAC. The biological activity of CD351 was evaluated in various *in vitro* 2D and 3D models, including BC cell lines (MDA-MB-231, MCF-7, and MCF-7R), a PDAC cell line (PANC-1), and non-tumoral cells such as human mammary epithelial cells (HMEpC) and human dermal fibroblasts (HDF). Additionally, the *in vivo* toxicity of CD351 was assessed in C57BL/6 mouse models. In 2D cell cultures, CD351 exhibited potent photodynamic activity, with IC_{50} values in the nanomolar range. In 3D tumor models, CD351 showed high efficacy in TNBC spheroids at concentrations as low as 50 nM. Furthermore, in a complex PDAC heterotypic spheroid model (STAM), CD351 reduced tumor viability by approximately 50%, indicating strong activity even in physiologically relevant microenvironments. Preliminary *in vivo* toxicity assessments in C57BL/6 mice revealed no observable adverse effects. The findings of this study will be discussed in detail during the presentation.

Funding/Acknowledgements: This research work received financial support from the University of Aveiro and FCT/MCTES to support the UID/50006 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos research unit, the Institute for Biomedicine – iBiMED (UIDB/50011/2020 & UIDP/50011/2020) and CICECO-Aveiro Institute of Materials (UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020) and LAQV-REQUIMTE (UIDB/50006/2020), financed by national funds through the FCT/MCTES (PIDDAC).

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Singlet molecular oxygen in biological systems: Mechanistic studies using ^{18}O-labeled dioxygen, mass spectrometry, and near-infrared light emission measurements

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Keywords: singlet oxygen; ^{18}O-labeled oxygen; mass spectrometry; light emission

Evidence has accumulated during the past last three decades regarding the strong involvement of several oxidants including hydroxyl radical, one-electron oxidants and singlet molecular oxygen [$^1O_2$] in the generation of hydroperoxides from various nucleobases, amino acids and unsaturated lipid components. Our focus lies in elucidating the reaction mechanisms and the characterizing the products formed from the reaction of specific oxidants, $^1O_2$ and peroxides, with defined biomolecules, as well as in the developing robust and sensitive methodologies to detect these products in biological samples [1]. The measurement of near-infrared (NIR) light emission, along with the use of ^{18}O-labeled $^1O_2$ and hydroperoxides generated via a clean naphthalene-derived thermolabile endoperoxide (DHPN$^{18}O_2$) serves as a powerful tool for studying mechanistic aspects of $^1O_2$ generation and reactivity in biological systems [1]. Participation in an international network led to the first demonstration of $^1O_2$ and peroxide involvement in mammal physiology [2]. In irradiated biological system, UVA photons are poorly absorbed by the DNA and are more significantly absorbed by other cellular chromophores [3,4]. We present the direct spectroscopic detection and characterization of $^1O_2$ NIR monomol light emission from 6-thioguanine exposed to UVA light. Methionine is one of the main targets for biological oxidants. Its reaction with most oxidants generates only methionine sulfoxide. However, when N-terminal methionine reacts with hypohalous acids (HOCl and HOBr) or $^1O_2$, it can also form a cyclic product called dehydromethionine (DHM). Preliminary studies are being undertaken to evaluate the role of $^1O_2$ in activated neutrophils and eosinophils [5, 6]. Despite generating DHM, reaction of $^1O_2$ with tryptophan and tyrosine (either free or inserted in proteins) generates hydroperoxides [7]. Other sources of $^1O_2$ that have been developed include photosensitizers excited by upconversion nanoparticles and Color-Tunable CdSe/ZnS Nanocrystals [8, 9]. The approach used to unequivocally demonstrate the generation of $^1O_2$ in these reactions involves the use of ^{18}O-labeled hydroperoxide/triplet dioxygen ($^{18}[^3O_2]$), the detection of labeled compounds by HPLC coupled to mass spectrometry (HPLC-MS^n) and the direct spectroscopic detection and characterization of $^1O_2$ NIR light emission. The combined use of thermolysis of a water-soluble naphthalene endoperoxide as a generator of ^{18}O-labeled $^1O_2$ enabled the study of $^1O_2$ reactivity toward biomolecules. Photoemission properties and chemical trapping clearly demonstrate that the production of hydroperoxides and excited carbonyls [10] generates $^{18}[^1O_2]$, and points to the involvement of $^1O_2$ in physiological and pathophysiological mechanism.

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Financial support: “Fundação de Amparo à Pesquisa do Estado de São Paulo” (FAPESP) CEPID Redoxoma 2013/07937–8; “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) Grants 304350/2023-0 and 304945/2021-8; “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES); Universidade de São Paulo (USP)” NAP Redoxoma (PRPUSP 2011.1.9352.1.8) and John Simon Guggenheim Memorial Foundation (PDM).

Unveiling the Hidden Architecture of the Brain: the Power of Super-Resolution Shadow Imaging and Optogenetics

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Keywords: Optogenetics, Shadow Imaging, STED, Neuroscience

Introduction

This work seeks to illuminate the role of the extracellular space (ECS) in neuronal signaling by leveraging the innovative Super-Resolution Shadow Imaging (SUSHI) [1] technique alongside optogenetic tools. These cutting-edge approaches enable nanoscale mapping of the ECS across entire mouse brain hemispheres, offering a unique perspective on its structure and dynamics. By rendering all cells as analyzable shadows, this method generates detailed ECS maps that complement existing cellular maps—clarifying the spatial and temporal patterns of ECS behavior and their connection to extracellular matrix protein organization. More broadly, this research aims to advance our understanding of brain microarchitecture and its dynamic complexity. Central to my focus is uncovering how disruptions in this finely tuned system may underlie the development and progression of neurological disorders.

Materials and methods

- Animal Preparation: Organotypic brain slice cultures were prepared from postnatal day 6–8 (P6–P8) mouse pups. Coronal brain slices (250 µm thick) were prepared using a comprestome-microtome (VF 500 0Z) and then placed onto coverslips in rollerdrum tubes containing culture medium. Slices were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 1–2 weeks to allow stabilization and preservation of native tissue architecture, including the extracellular space (ECS). Media was changed every once per week.
- Super-Resolution Shadow Imaging (SUSHI): SUSHI was employed to map the extracellular space (ECS) at nanoscale resolution. This technique involves the use of fluorescent labeling of the extracellular fluid to render all cellular components as dark "shadows" against a bright ECS background. Brain slices were stained with a membrane-impermeant fluorescent dye, which diffuses into the ECS. Imaging was conducted using a custom-built super-resolution microscope, combining stimulated emission depletion (STED)2.
- Optogenetic Stimulation: To manipulate neuronal activity and probe dynamic ECS changes, slices were virally transduced with AAV vectors carrying ChR2 3 or cytoskeletal modulators under a neuron-specific promoter. Light stimulation (470 nm) was applied using a programmable LED system coupled to the imaging setup. Temporal parameters of stimulation were optimized to mimic physiologically relevant activity patterns.

Results

Using the Super-Resolution Shadow Imaging (SUSHI) technique on organotypic brain slices from neonatal mice, we generated high-resolution, three-dimensional maps of the extracellular space (ECS) across large tissue volumes. The SUSHI approach effectively rendered all cellular components as optically negative shadows, enabling clear visualization and quantification of ECS morphology with nanoscale precision. Our data revealed that the ECS is highly heterogeneous in both structure and dynamics, even within morphologically similar brain regions. Dynamic imaging in live slices showed that ECS volume fraction and diffusion properties fluctuate over time, correlating with patterns of neuronal activity induced through optogenetic stimulation. Notably, light-induced activation of excitatory neurons led to transient ECS shrinkage and altered diffusion kinetics, suggesting a tight coupling between network activity and extracellular microarchitecture. Further analysis demonstrated that areas with denser extracellular matrix protein labeling exhibited reduced ECS volume and more restricted diffusion, indicating that ECM organization contributes to ECS compartmentalization. These findings support the notion that ECS is not a passive medium but a structurally dynamic environment that responds to and potentially regulates neuronal activity.

Conclusions

This study highlights the power of combining SUSHI and optogenetics to investigate the extracellular space in intact brain tissue. Our results demonstrate that the ECS is a dynamic, structurally diverse compartment influenced by both neuronal activity and extracellular matrix organization. These insights challenge traditional views of the ECS as a static diffusion space and underscore its potential role in modulating neurophysiological processes.

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Investigating the Impact of Blue Light and UVB on Skin DNA Repair and Pigmentation: In Vitro and In Vivo Insights

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Ultraviolet (UV) irradiation is known to cause harmful effects, including skin aging and skin cancer, due to the increased production of cellular reactive species and direct DNA damage. Alongside UV, blue light (BL), particularly high-energy visible light (400–450 nm), contributes to skin photoaging by triggering the production of free radicals, reactive oxygen and nitrogen species. It is estimated that up to 50% of free radicals produced in the skin result from visible light exposure. BL has been found to act synergistically with UVA to induce skin pigmentation, thereby eliciting or exacerbating hyperpigmentation disorders such as post-inflammatory hyperpigmentation and melasma, especially in individuals with darker skin tones and induced 8-oxoguanine lesions formation within DNA, which are indicative of oxidative stress and can contribute to mutagenesis and cellular dysfunction. However, data on the synergistic detrimental effects of BL and UV are limited. Our study aims to evaluate the effects induced by BL exposure followed by UVB exposure. We investigated this impact on the repair of pyrimidine dimers, the primary class of premutagenic DNA damage caused by sunlight exposure, and hyperpigmentation parameters.

For the in vitro study, reconstructed human epidermis (RHE) was first exposed to BL and then to UVB. SPF50+ sunscreen formulations containing a new broad-screen filter were applied at a dose of 2 mg/cm² on polymethyl methacrylate plate, which was then placed on the RHE model before BL exposure. DNA was extracted and analyzed by liquid chromatography/tandem mass spectrometry (LC-MS/MS) to quantify DNA lesions.

For the clinical study, we enrolled 27 healthy female and male volunteers with phototypes III and IV (50/50). Five zones were identified and randomized on the volunteers' backs: BL (60J/cm², 412±5nm), UVB (150mJ/cm², 312 nm), BL/UVB, BL Photoprotection + BL/UVB, and a negative control zone. Chromametric measurements were performed and skin biopsies of 4mm Ø were taken from the investigational zones for DNA lesion quantification. DNA was extracted and analysed by LC-MS/MS. Pyrimidine dimers were not detected by BL exposure alone but exposure of RHE to environmentally relevant doses of BL significantly decreased the repair rate of cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts induced by subsequent UVB irradiation. Moreover, we found that applying a SPF50+ sunscreen during BL exposure prevented DNA decrease repair efficiency. Increase of the pigmentation was observed on volunteers regardless of phototype following BL exposure. Those with greater pigmentation also showed enhanced sensitivity in their DNA repair systems, resulting in a higher number of residual DNA lesions. When SPF50+ sunscreen was applied, the adverse effects of BL on pigmentation and the DNA lesion repair system were drastically reduced.

Our findings demonstrate that BL impairs the repair of pyrimidine dimers in the DNA of skin models and healthy volunteers, and that BL exposure through an SPF50+ sunscreen specifically prevents a decrease in repair capacities.

Blue Light from VR-HMDs: Unanswered Questions and the Need for Research

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Keywords: Blue Light Hazard; Virtual Reality; Retina; LED

Background: Digitalization and the broad spectrum of potential applications have led to an ongoing increase in the use of virtual reality (VR) technologies in recent years. VR head-mounted displays (HMDs) utilize light-emitting diodes (LEDs) and expose the eyes of the users to visible light (400 nm - 780 nm) at extremely close range, often less than 15 mm. Furthermore, it is well documented that acute exposure to high-intensity blue light (400 nm - 500 nm) can lead to photochemical damage in the lens and retina. However, it remains unknown whether long-term exposure to blue light from VR-HMDs can trigger or contribute to photochemical effects in the eye.

Methods: We conducted a literature search with the search terms “blue light”, “retina”, “eye”, “virtual reality”, “light emitting diode”, “exposure”, “in vitro” and “in vivo” in various combinations. Our goal was to identify, analyze and summarize both review and original articles examining the effects of blue light exposure from LEDs on the retina.

Results: As demonstrated by a substantial body of research, exposure to high-intensity blue light from LEDs has been shown to induce a number of biological responses, including oxidative stress, apoptosis and necroptosis, disruption of mitochondrial fission and fusion processes, and inflammation in both *in vitro* and *in vivo*. However, existing research cannot be reliably used to estimate the effects of blue light exposure from VR-HMDs for a multitude of reasons. 1) *In vitro* biological models in which human retinal pigment epithelial cells are directly irradiated, do not adequately mimic the structures of the human eye and retina. 2) *In vivo* studies with rodents do not take anatomical differences between the rodent and human retina into account. 3) Considerable variations are observed in the emission spectra and photometric specifications of used light sources and exposure scenarios vary considerably between studies. This discrepancy in experimental conditions renders a systematic comparison of study results virtually unfeasible. 4) Exposure studies with blue light irradiance levels similar to the exposure conditions occurring with the use of VR-HMDs have not yet been conducted.

Conclusions: Given the increasing use of VR-HMDs and the premise that these devices could be worn for eight or more hours daily, the question arises as to whether the prolonged use of VR-HMDs may contribute to or cause photochemical damage to the retina. As a first step toward answering this question, we propose to investigate, whether previously described photochemical effects of blue light in the retina can also occur under exposure conditions like those when using VR-HMDs. The overarching goal of the investigation should be to develop a feasible and reproducible biological model of the human retina and to implement a standardized exposure system for assessing the photochemical and photobiological effects on the retina of emerging light-based technologies.

Characterisation of urinary thymidine dimer excretion in hairless mice exposed to ultraviolet radiation

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Keywords: cyclobutane pyrimidine dimers; DNA repair; biomarker; non-invasive screening

Development of skin cancer is strongly associated with mutations caused by ultraviolet radiation (UVR). Exposure to UVR induces the formation of DNA damage such as cyclobutane pyrimidine dimers (CPDs) that must be repaired by the nucleotide excision repair pathway. We have previously shown that the predominant type of CPDs, thymidine dimers (T<>T), can be detected in human urine using liquid chromatography coupled to mass spectrometry (LC-MS) [1,2]. We report that T<>Ts can also be detected in urine from hairless mice exposed to UVR [3]. The study set out to characterise the urinary excretion of T<>Ts in hairless mice by determining the dose-response and excretion kinetics, the impact of different UVR sources and delivery durations, and the effect of acute immunosuppressant treatment on T<>T excretion. Hairless mice were exposed to increasing doses of UVR (0.125-3.5 standard erythema doses (SEDs)), and urine was collected for LC-MS analysis. T<>Ts could be detected in mice exposed to ≥ 0.25 SEDs, but only 3.5 SEDs resulted in erythema. A linear correlation was found between UVR dose and T<>T concentration on the day after UVR ($r^2 = 0.9952$; $p < 0.01$). Excretion of T<>T was detected already 80 minutes after UVR with an increase in concentration over time. Exposure to pure UVA irradiation (2 SEDs at 365 nm) also induced the excretion of T<>Ts but significantly less compared to a UVB-rich UVR source ($p < 0.01$). Furthermore, the duration of exposure impacted T<>T excretion with 2 SEDs given over 1 hour resulting in a higher concentration compared to 6 hours ($p < 0.01$). Application of sunscreen (SPF 30) to hairless mice prevented the excretion of T<>T following 3.5 SEDs of UVR. In mice treated with immunosuppressants for two weeks, excretion of T<>T following UVR was reduced in the majority of treatment groups ($p < 0.05$). Overall, our data point to the use of thymidine dimer excretion as a new, non-invasive biomarker in the screening and evaluation of new preventative therapies or photocarcinogens.

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Photoactive MDM2 inhibitors: light related strategies to tackle hard to treat cancers

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Keywords: Photoactivatable; anticancer; photoswitchable; protein-protein interaction inhibitor

Tumoral cases among the population have been steadily increasing, and the treatment of cancer remains a major challenge. Due to the large number of chemotherapeutic agents employed and the frequent occurrence of relapses, patient life expectancy is often severely compromised. [1]

Targeting protein–protein interactions (PPIs) with high spatiotemporal precision remains a major challenge in drug discovery and chemical biology. The oncoprotein MDM2, a key negative regulator of the tumor suppressor p53, is an especially attractive target due to its central role in cancer progression and its structurally well-defined binding pocket. Conventional MDM2 inhibitors, which aim to restore the tumor-suppressive function of p53, show promise but suffer from systemic toxicity due to p53 activation in healthy tissues. To overcome this limitation, we explore **light-activated pharmacological approaches such as photoprotective groups or photoswitches,** for achieving spatiotemporal control over the MDM2–p53 interaction. [1] In this approach, the inhibitors remain inactive in the absence of light and only become functionally active as anticancer agents upon exposure to visible light [Fig 1.]. To improve clinical relevance, we have designed and synthesized novel compounds that are **photoactive in the visible-light range**, enabling deeper tissue penetration and reduced phototoxicity. Through the fine-tuning of the compounds the activation occurs at different light sources such as blue, green and red light. We will show our most recent results on blocking MDM2-p53 interaction with visible light-activated small molecules.

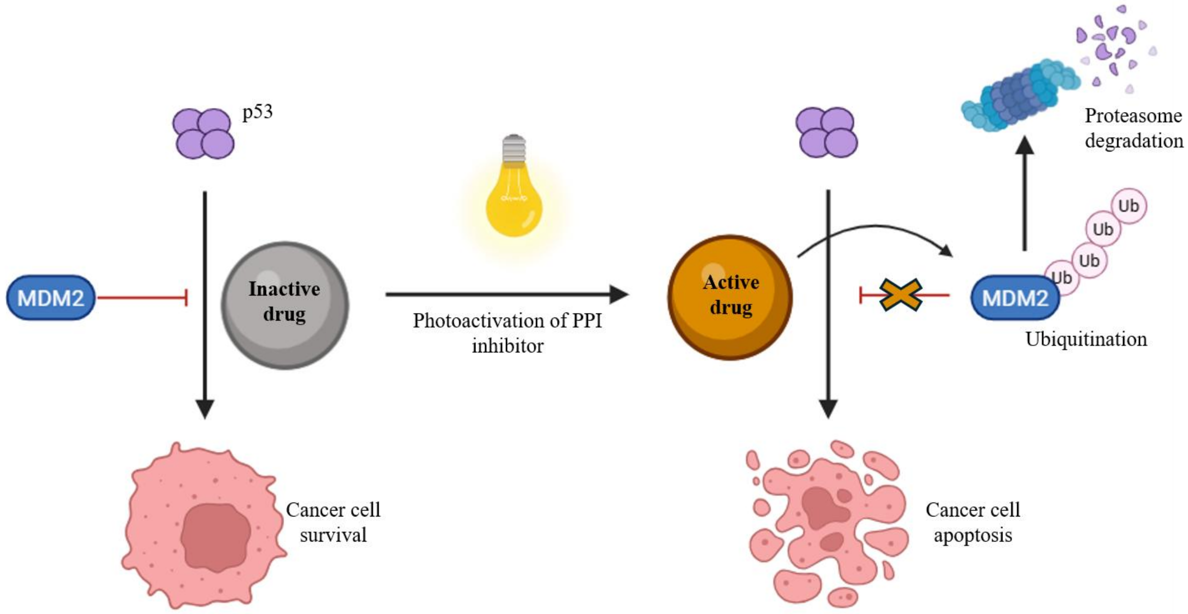

Fig1. Schematic representation of activation of photoactivatable PPI inhibitor

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Effects of CuInZnS/ZnS quantum dots on microalgae at different environment conditions

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Keywords: nanoparticles; aqueous media; autofluorescence; microscopy.

The effects of quantum dots (QDs), including CuInZnS/ZnS-COOH (Cu-based) QDs, on microalgae are a subject of growing scientific interest, primarily due to the increasing presence of nanomaterials in aquatic environments. In essence, while Cu-based quantum dots may present a less toxic alternative compared to some other quantum dot types, they still pose potential risks to aquatic organisms and their impact on microalgae still warrants careful consideration and further research. Microalgae, as bioindicators, play a crucial role in assessing water quality and detecting pollutants due to their sensitivity to environmental changes and rapid physiological responses. The interaction of some types of nanoparticles with microalgae can disrupt vital cellular processes, such as photosynthesis [1]. Pulse-Amplitude modulated (PAM) fluorometry offers a reliable, non-invasive method for monitoring changes in microalgae autofluorescence and photosynthetic parameters. Combining Fluorescence (FL) and Coherent Anti-Stokes Raman Scattering (CARS) [2] microscopy techniques provide very detailed information, for example, the distribution of natural chemicals within the microalgae, and how those distributions are effected by the quantum dots. However, there is limited understanding of how the different types of QDs vary depending on algae species and how environmental conditions influence these effects.

The aim of this study is to evaluate the interaction of CuInZnS/ZnS-COOH QDs with *Desmodesmus sp.* and *Scenedesmus sp.*, comparing specific QD-induced responses on photosynthetic efficiency and overall physiology at different environment conditions. Cu-based QDs in *Scenedesmus sp.* and *Desmodesmus sp.* algae disrupt the photoadaptation process: however, the specific changes detected depend on the growth conditions to which the algae have been adapted. Cu-based QDs cause a decrease in ETR and Y(II) of *Scenedesmus sp.* algae, but the change in non-photochemical coefficients depends on the algae's adaptation to environmental conditions. Cu-based quantum dots induce cell death of *Desmodesmus sp.* algae: in the initial stage, decrease in autofluorescence and the CARS signal from carotenoids was registered after 24 h the; and after 48 h fluorescence signal of chlorophyll released from the algae were determined.

The knowledge of the ecotoxicity of different types of quantum dots on a wider range of microalgae species would not only help to predict and mitigate the extent of possible damage, caused by QDs when entering the ecosystem, but would also help in the development and improvement of QDs use in industry and medicine.

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Sunscreens: Yet another plea for a neutral grey filter

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Keywords: sunscreen; UV-A; skin cancer

In many places, ambient available UV is more abundant than human skin can sustain. Therefore, we seek shade, put on clothes and use sunscreen. The bottle of the latter promises to reduce skin redness, which is predominantly a UVB effect. On the other hand, we advise the use of sunscreen to prevent skin cancer, which is not an effect claimed by the producers, and for decades now, UVA has been recognized to contribute to human skin carcinogenesis [1,2,3,4].

Still the EU only suggests to protect UVA with a factor 1/3 of the UVB protection. Discriminating information for the quality of the UVA filter is not available on the label and consumers cannot make an informed choice in the shop. Why is this? Does it make sense? Where do the cells at risk for skin cancer actually reside [5,6,7,8,9,10]? What consequences does this information have? In fact, from all health effects, the best sunscreen should offer the least protection against erythema, whereas today it is optimized for this endpoint. Erythema is your smoke detector: Don't switch it off! We review what is known about this issue today and translate this updated knowledge into an updated advise for sunscreen formulation, labelling and EU regulations.

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Depigmenting Action and Bioimaging of Luminescent Piperazinyl Phenol Derivatives

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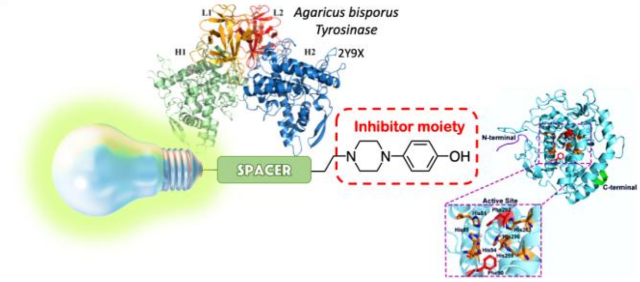
Keywords: Melanogenesis; Tyrosinase; Chromophores; Bioimaging; Depigmentation

Melanogenesis is a complex enzymatic process in which Tyrosinase (TYR, EC 1.14.18.1) catalyzes the conversion of L-DOPA into dopaquinone, which is in turn converted in several steps into melanin.[1] This process is involved in various skin diseases, including vitiligo (hypopigmentation), melasma (hyperpigmentation) [2] and in the onset of melanomas as well as in neurodegenerative diseases such as Parkinson and Alzheimer.[3] The approaches used to inhibit melanogenesis are different: enzymatic regulation, gene regulation through transcription factors or signaling.[1] In general, the control of TYR level represents the most followed approach, also because it is strictly related to the onset of these pathologies and can be considered an important biomarker. Therefore, the detection of TYR with high sensitivity is of great importance to study its activity in biological processes as well as in clinical diagnosis.[3] Finally, the possibility of inhibiting TYR is useful in the medical field to treat disorders and diseases associated with melanin accumulation. Synthetic and natural TYR inhibitors have been identified in last years,[4] but the clinical application of these substances is often limited for their degradation and consequently poor bioavailability, and in some cases for problems of toxicity or side effects. Among the synthetic inhibitors, we focused our attention on 4-(1-piperazinyl)phenol derivatives [5] that showed *in silico* and *in vitro* inhibitory activity towards the TYR of *Agaricus bisporus* (AbTyr). In this work, we developed a novel series of luminescent derivatives of 4-(1-piperazinyl)phenol, obtained by covalently linking key chromophores such as BODIPY, Curcumin, and Porphyrin to the core scaffold. These modifications were designed to combine biological activity with tailored photophysical properties. The compounds were evaluated through a multidisciplinary approach involving *in silico* modeling, *in vitro* assays, and *in vivo* testing. We will present the most relevant results, focusing on compounds showing dual functionality, as potential bioimaging probes and as depigmenting agents, highlighting their innovative role at the interface between medicinal chemistry and molecular imaging.



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Cellular Photodamage Induced by Dasatinib: Type I/II Mechanisms and Photoprotection in Reconstructed Epidermis

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Keywords: Artificial skin, Keratinocyte cells, Tyrosine kinase inhibitor, Ultraviolet filter

Dasatinib (DAS) is an anticancer drug used to treat hematological malignancies, such as chronic myeloid leukemia and Philadelphia chromosome positive acute lymphoblastic leukemia [1,2]. While initially developed for oral administration, DAS has recently garnered interest in topical application. However, topical drugs may induce photosensitivity, an adverse effect not currently listed for DAS [3,4]. Given its UVA absorbing chromophore, DAS may trigger phototoxic reactions via Type I mechanisms mediated by free radicals and Type II mechanisms mediated by singlet oxygen. This study investigated DAS induced phototoxicity in solution and reconstructed human epidermis, mimicking topical exposure. Upon UVA irradiation, DAS generates a triplet excited state (^{3}DAS*) that leads to the production of reactive oxygen species (ROS) and organic radicals. In vitro assays, including NRU, comet assay, lipid peroxidation (C11-BODIPY), protein carbonylation, and DNA oxidation, confirmed photo(geno)toxicity to cells. These results suggest that various cellular compartments are potential targets of oxidative damage mediated by DAS. Significant phototoxicity was also observed in reconstructed epidermis. Notably, this effect was significantly reduced when DAS was combined with a UVA filter (Tinosorb S), indicating an effective photoprotection strategy for the safe development of topical DAS formulations. [5].

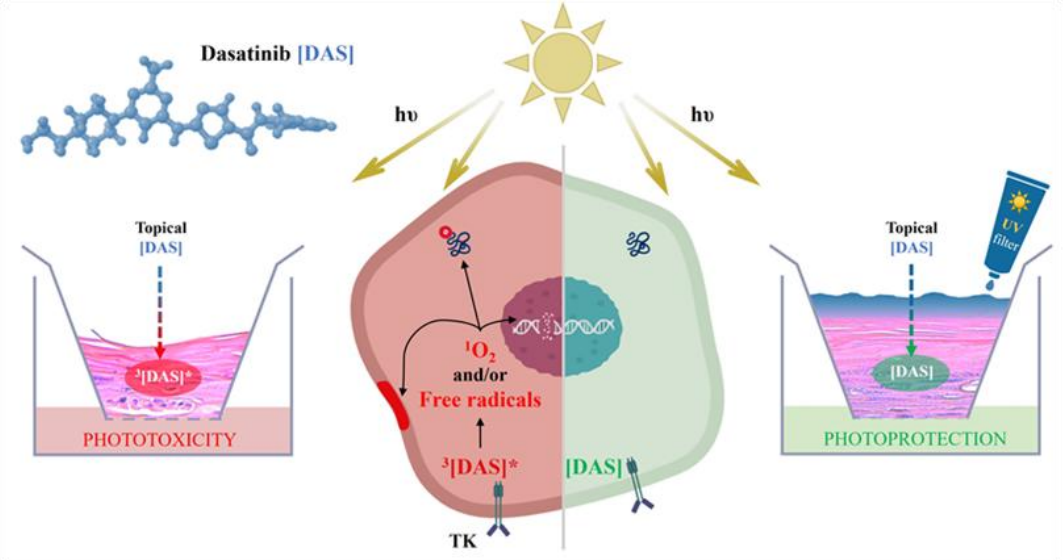

Figure. A schematic illustration showing that DAS phototoxicity may be prevented by including sunscreen in its topical formulation.

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Carbon quantum dots as the latest component of sunscreen products to protect the skin from blue light

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Keywords: blue light protection; carbon quantum dots; antioxidants; sunscreen product

In recent years, there has been growing concern about the harmful effects on the skin of not only UV radiation, but also blue light, also known as high-energy visible light (HEVL) with wavelengths in the 400-500 nm range. This light penetrates deep into the dermis, causing oxidative stress, hyperpigmentation, and accelerated photoaging. Today, more and more consumers are looking for sunscreens not only with a high SPF level, but also with additional protection against blue light. However, since most new chemical UV filters have limited effectiveness within this wavelength range, this necessitates the development of new functional materials to reliably protect the skin from blue light. Carbon quantum dots (CQDs) are a new class of materials with a particle size of less than 10 nm, characterized by high photocatalytic properties, biocompatibility, and low toxicity, and can be considered as promising candidates for research on skin protection against blue light.

The aim of the work is to synthesize CQDs and study their optical and antioxidant properties to assess the feasibility of their use as an active component in cosmetic sunscreen products to protect the skin from blue light.

Materials and methods. CQDs were synthesized by the microwave method using urea and citric acid (Merk, USA) in a ratio of 8:6, respectively. The optical properties of CQDs were studied using a Varioskan Flash multifunctional plate analyzer (Thermo Scientific, USA). The light transmission by CQDs solutions in glycerin at concentrations of 1, 5, 10, and 15% in the spectral range of 400-450 nm was studied using a Labsphere UV-1000 Ultraviolet Transmittance Analyzer (Labsphere, USA) on PMMA Sunplates MD4-5 molded (HelioScience, France). Based on the obtained transmittance spectra for each concentration of CQDs, the area under the transmittance curves in a given range was calculated. In addition, CQDs were introduced into a model sunscreen based on chemical filters with SPF 30 to assess their contribution to improving protection in the 400-450 nm range. The antioxidant properties of CQDs were also investigated *in vitro* spectrophotometrically on Specord 200 Plus (Analytik Jena, Germany) in the *redox* system of auto-oxidation of adrenaline.

Results. CQDs have been synthesized, which under UV irradiation are characterized by green fluorescence, and the maximum optical absorption is observed at a wavelength of 336 nm. As a result of the spectral analysis of the fluorescence of the synthesized CQDs, it was found that the maximum emission peak is observed at a wavelength of 478 nm under excitation at 280 nm. The quantum yield of CQDs is 25.3 %, which is a rather high value indicating their efficiency as fluorescent materials. It was found that with an increase in the concentration of CQDs in a glycerin solution, the area under the transmission curve in the range of 400-450 nm decreases significantly: from 5000 conditional units (c.u.) for glycerin solution to 3936 c.u. for 1% CQDs, 3266 c.u. for 5% CQDs, 2774 c.u. for 10% CQDs, and 2492 c.u. for 15% CQDs. It was proved that the addition of 5% green CQDs to a sunscreen with SPF30 significantly improves its photoprotective properties in the range of 400-450 nm, which is manifested in a decrease in the area under the transmission curve from 4526 c.u. to 2867 c.u. There is also an increase in the critical wavelength from 375 nm to 380 nm. It was found that CQDs at a final concentration of 15 mg/l in the adrenaline auto-oxidation system slow down the reaction rate and, accordingly, the generation of superoxide radicals by 1.31 times: $K_n^1{}_{(0)}=(1.53\pm0.02)\times10^{-4}\ s^{-1}$ and $K_n^1{}_{(CQD\ 15\ mg/l)}=(1.17\pm0.03)\times10^{-4}\ s^{-1}$. Instead, when the concentration of CQDs doubled, the reaction rate decreased by 1.46 times, and at a concentration of 50 mg/l – by 1.63 times, indicating a dose-dependent antioxidant effect in this system: $K_n^1{}_{(CQD\ 30\ mg/l)}=(1.05\pm0.02)\times10^{-4}\ s^{-1}$ and $K_n^1{}_{(CQD\ 50\ mg/l)}=(0.94\pm0.05)\times10^{-4}\ s^{-1}$.

Conclusions. Due to their pronounced optical characteristics and antioxidant activity, CQDs with green fluorescence can be effectively used in cosmetic sunscreen products to protect the skin from blue light.

Predicting Singlet-Oxygen Quantum Yield in Porphyrinic and Non-Porphyrinic Photosensitizers via Novel Quantum Descriptors

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Keywords: Singlet-oxygen; Quantum-descriptors; Photosensitizers; Prediction

Reliable in silico prediction of singlet-oxygen quantum yield (Φ_Δ) is pivotal for developing next-generation phototherapeutics and photocatalysts. We present a ten-descriptor multiple-linear-regression model that achieves R = 0.87 (SD = 0.12) for Φ_Δ in toluene, trained and internally validated on 95 structurally diverse photosensitizers (porphyrinic and non-porphyrinic, with and without heavy atoms). This accuracy, coupled with consistent cross-fold stability, surpasses prior models[1,2] in both predictive performance and chemical generalizability while retaining full physicochemical interpretability.

Six descriptors are newly formulated here for the first time. Two of them quantify the key energetic and spin-orbital ingredients of intersystem crossing (ISC):

$$\mathbf{EIGTS} = e^{-1/|E_{T1}-E_{S1}|}, \qquad \mathbf{LCSOC} = \ln\Big(\sum_{t=1}^{5}\sum_{s=0}^{5}\big|\langle T_t\,|\,\hat{H}_{\mathrm{SO}}\,|\,S_s\rangle\big|\Big),$$

here E_{T1} and E_{S1} are adiabatic triplet and singlet energies, and $\hat{H}_{SO}$ is the spin–orbit Hamiltonian. **EIGTS** provides an exponentially weighted measure of the T1→S1 energy gap, while **LCSOC** condenses the cumulative spin–orbit-coupling landscape into a single logarithmic term.

These metrics integrate with (i) a frontier-orbital/reactivity block constructed through non-linear transforms and (ii) a compact topology–polarity signature that merges size, polarity and charge-dispersion effects. Together, the descriptors encode the interplay between excited-state dynamics and solvent-mediated charge redistribution.

Stepwise analysis identifies **EIGTS** and **LCSOC** as dominant positive contributors, confirming that an optimised T1–S1 gap and a robust cumulative SOC profile act synergistically to maximise Φ_Δ. With a prediction uncertainty of ±12 %—within experimental reproducibility— this model delivers higher accuracy, broader scaffold coverage, and clearer mechanistic insight than any previous Φ_Δ correlation, enabling confident pre-synthetic prioritization of photosensitizers. The fully automated Python workflow scales seamlessly to high-throughput virtual screening, demonstrating the power of these previously unreported quantum-chemical descriptors for accelerating the discovery of efficient singlet-oxygen generators.

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Synthesis and characterization of new purpurin imide derivatives with functional groups from Purpurin 18 for PDT application.

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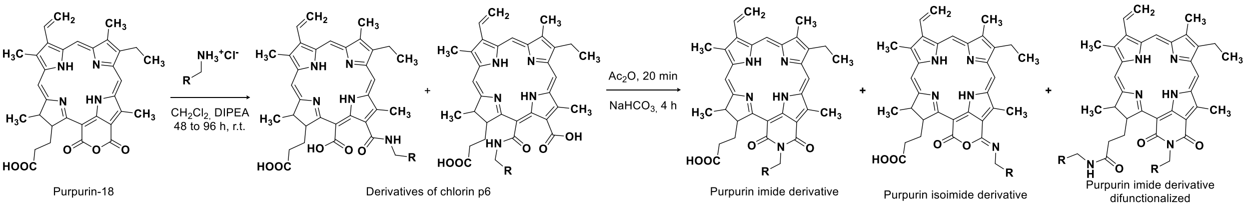
Keywords: Purpurin imide derivatives, chlorins, purpurin 18, photosensitizers

Chlorins are among the most interesting photosensitizers for photodynamic therapy because they exhibit a high quantum yield of the singlet oxygen generation and absorbance in the deep red. 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 acetone extraction of chlorophyll a from *Spirulina 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 660 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 works 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. In this work, we have therefore synthesized various derivatives of purpurin imide, bearing different peripherical 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 purpurin imide were characterized by NMR, UV/visible spectroscopy and mass spectrometry [2]. Preliminary data of fluorescence and singlet oxygen production will be discuss. One example of use for PDT application on colorectal cancer cell line (HCT116 and HT 29) [3].



General synthesis of Purpurin imide derivatives from Purpurin 18

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Supramolecular Interactions Between Cationic Porphyrins and Anionic Carboxymethyl cellulose

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Keywords: porphyrins, carboxymethilcellulose, electrostatic interaction, sensing

Carboxymethyl cellulose (CMC), a derivative of cellulose, has gained increasing attention as a highly versatile polymer due to its wide range of functional properties.[1] CMC acts as an effective chelating agent, capable of binding metal ions and facilitating their removal from aqueous solutions. Its structure, enriched with carboxymethyl groups, imparts a high adsorption capacity, making it particularly suitable for the sequestration of heavy metal pollutants such as copper, nickel, and zinc from water sources.[2]

In this study, we explored the non-covalent interactions between CMC and 5,15-bis(1-methylpyridinium-4-yl)-10,20-diphenylporphyrin (t-H_2Pagg), along with its metal complexes, with the aim of developing novel molecular sensing platforms for heavy metal detection. Spectroscopic analyses including UV/Vis absorption, fluorescence, resonant light scattering (RLS), and circular dichroism (CD) revealed that the binding affinity between CMC and the porphyrin derivatives is significantly influenced by the nature of the metal and its coordination geometry.[3]

These selective and tunable interactions offer promising opportunities for the development of fluorometric sensors capable of detecting target metals in complex environments such as contaminated water. Moreover, the supramolecular recognition abilities of CMC–porphyrin systems could potentially be extended to biological targets, such as DNA, paving the way for innovative applications in biosensing

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Cyanobacteria as Living Biohybrids: Porous PEDOT:PSS Interfaces for Environmental Electrophysiology

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Keywords: Bioelectricity; Cyanobacteria; porous PEDOT:PSS electrodes; Electrochemical Impedance Spectroscopy

Cyanobacteria have long played—and continue to play—a pivotal role in Earth's evolutionary and ecological systems. In recent decades, however, certain strains—particularly those producing taste and odour (T&O) metabolites and toxins—have become a growing concern for human health and drinking water safety. Current monitoring strategies focus primarily on chemical (e.g., nitrogen, phosphorus) and environmental (e.g., temperature, dissolved oxygen) parameters, or indirect biological proxies such as chlorophyll-a. These methods, however, lack the capacity for real-time detection and early warning.

In this talk, I present a novel approach to cyanobacteria sensing by demonstrating that cohorts of the problematic species *Oscillatoria* sp. exhibit electrical excitability, governed largely by mass diffusion processes. This bioelectric activity—mediated by paracrine signalling—appears to facilitate rapid collective stress acclimation [1]. The underlying measurements are enabled by an ultrasensitive electrophysiology platform based on porous polyurethane (PU) foams coated with PEDOT:PSS, leveraging ultra-large-area electrodes to maximize double-layer capacitance and signal sensitivity [2]. Microbial viability and growth are tracked in real-time via Electrochemical Impedance Spectroscopy (EIS) and compared with standard analytical methods for cell counting and equivalent circuit modelling [3]. The cumulative signal scales with electrode area, indicating a correlation with biomass and productivity. Furthermore, we interpret cooperative signalling as Ca^{2+} waves, validated via fluorescence imaging, and suppressed using gadolinium chloride, a specific ion channel inhibitor.

This work suggests that decoding the electrogenic dialogue of cyanobacteria offers a path toward more efficient and preventative water quality monitoring, and illustrates the potential of biohybrid electronics in environmental sensing.

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Photosynthetic biohybrids for sensing, current generation, and solar fuels

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Keywords: photosynthetic bacteria; photobioelectrochemistry; biophotoelectrodes; hydrogen; redox polymers; nanomaterials

Photosynthetic bacteria are of great interest for the development of biohybrid systems thanks to their capability to convert solar energy into electrical and chemical energy. By coupling intact cells of photosynthetic microorganisms to electrodes it is possible to obtain photo-bioelectrocatalysts that can be employed to perform the sensing of environmental pollutants, the localized generation of photocurrent, or the synthesis of solar fuels.[1, 2]

Anoxygenic purple bacteria have been explored for all the aforementioned applications as they can utilize organic contaminants present in a solution as carbon sources while using sunlight as energy source, thus enabling complex reactions to take place in an elegant fashion.[3]

A critical challenge affecting these biohybrid is the limited current density resulting from the hindered photo-induced electron transfer, which in turn limits sensitivity of the sensors, current densities, and product yields. The talk will present the most recent approaches that we have undertaken to tackle this challenge, with the implementation of biocompatible polymers and inorganic nanomaterials towards facilitating the extracellular electron transfer process.[4, 5] Future research needs and remaining challenges will also be discussed.

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Combining microbe and electrode modifications for improved biophotovoltaic performances.

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Keywords: biophotovoltaics, polypyrrole, polydopamine, cyanobacteria

Whole-cell biophotovoltaic (BPV) devices utilize photoautotrophic microorganisms like cyanobacteria for clean bioelectricity production, making them valuable assets in the energy transition. However, their performance remains limited by a key bottleneck: the inefficient charge transfer at the bioanode interface. Various strategies are currently being explored to overcome this challenge.

This study investigated the integration of separately engineered polypyrrole (PPy)-coated graphite electrodes [1, 2] with polydopamine (PDA)-modified *Synechocystis* sp. PCC6803 (*Synechocystis*) [1, 3] and aimed at enhancing photocurrent output through their complementary properties [1, 4].

Raman spectroscopy and atomic force microscopy were employed to characterize the PPy coating, while zeta potential and scanning electron microscopy provided insights into the cell–surface interactions. These findings were correlated with bioanode performance assessed via chronoamperometry.

The PPy coating on graphite was shown to effectively increase photocurrent when interfaced with *Synechocystis* under mediated conditions, indicating its potential as a support for PDA-modified microorganisms. The PDA-coated cells exhibited similar surface charge and adhesion to the PPy substrate as their uncoated counterparts, with an additive enhancement in photocurrent of ca. 40 % for the PDA-coated cells. These findings align with previous hypotheses suggesting that electrostatic interactions play a crucial role in cell adhesion to PPy [1, 2], with the increased photocurrent for PDA-decorated cells largely resulting from enhanced cell adherence on unmodified graphite electrodes [1, 3].

Overall, this work underscores the importance of early-stage compatibility assessments between biotic and abiotic elements in the development of BPV bioanodes - an essential step toward the effective deployment of full-scale BPV devices [1, 4].

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Enhancing Photosynthetic Bacteria-based Biohybrid Electrodes with Functionalized CeO_2 Nanoparticles

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Keywords: Biophotoelectrochemistry; *Rhodobacter capsulatus*; Cerium oxide; ROS scavenging

Microbial electrochemical systems (MES) have emerged as promising platforms for sustainable energy production and self-powered biosensing technologies by exploiting the metabolic activity of living microorganisms to generate electrical signals[1][2]. Our previous work demonstrated that modification of the photosynthetic bacterium *Rhodobacter capsulatus* with cysteine-coated gold nanoparticles (Au@cys) enhanced extracellular electron transfer (EET), resulting in increased photocurrent generation[3]. However, this improvement was transient, with significant drops in current density observed over time, likely due to intracellular accumulation of reactive oxygen species (ROS), which compromises cell integrity and long-term system performance.

To mitigate ROS-induced stress, we investigated the use of cerium oxide nanoparticles (CeO_2 NPs), well-known for their redox-active surfaces and ROS-scavenging capabilities[4]. Citrate (CeO_2@cit), aspartate (CeO_2@asp), and a combination of the two (CeO_2@mix) were used to functionalize CeO_2 nanoparticles. Low molecular weight hydrophilic ligands are chosen to enhance colloidal stability and improve interactions with the bacterial membranes where the photosynthetic apparatus is located. The antioxidant activity of the functionalized nanomaterials was quantified by two different radical scavenging assays, confirming their potential to reduce oxidative stress.

We systematically evaluated the impact of increasing nanoparticle concentrations and co-localization of CeO_2 and Au@cys nanoparticles on photocurrent generation and stability under continuous illumination. Our results compare differently functionalized CeO_2 nanoparticles in preserving microbial viability and sustaining photocurrent generation, offering a strategic approach to improve the longevity and reliability of self-powered bioelectrochemical devices.

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Biohybrid photoconverters from photosynthetic bacterial Reaction Centres and organic light harvesting dyes

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Keywords: Photosynthetic bacteria; Bioconjugation; Organic light harvesting antennas; Reaction Center photoenzymes.

The Reaction Center is an extremely efficient protein used by photosynthetic organisms, such as the *Rhodobacter sphaeroides* R26 bacterium, to convert solar energy and fuel their metabolism [1]. RC represents the sole photoenzyme in the biosphere employing sunlight to generate charge separated states with almost unitary conversion efficiency. Hence, it represents an ideal building-block for artificial biomimetic systems exploiting visible light to produce electrical signals. However, the applicability of RC in bioelectronic devices is related to employing chemical strategies to enhance the protein visible light absorption [2]. In fact, the RC light harvesting ability in the visible range is quite limited. Here we report our results showing that covalent bioconjugation of *Rh. sphaeroides* RC photoenzyme with tailored organic light harvesting molecules leads to biohybrid systems that not only retain the functionalities of the natural protein but also have enhanced photoconversion efficiency in the visible range, where the native RC is normally inactive [3,4]. We also demonstrate that, by properly tailoring the chemical structure of organic antenna molecules, it is also possible to modulate their light harvesting capabilities depending on various parameters such as pH, thus allowing photoadaptation of biohybrid systems to their working environmental conditions.

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Hybrid Microalgae-based Photoelectrodes for Solar Energy-Assisted Catalytic Conversions

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Keywords: microalgae; polydopamine; CO_2 reduction; hybrid catalysts.

Photosynthetic microorganisms are promising candidates for sustainable energy production in photobioelectrochemical systems. However, integrating them with electrodes is challenging due to the compartmentalized nature of photosynthetic organelles. Microalgae, in particular, have a complex cell structure, leading to low electron transfer rates and compromising electrochemical communication. Despite this challenge, microalgae are still considered a promising microorganism for such applications due to several advantages (photosynthetic efficiency, growth rate, mechanism against oxidative stress, non-production of toxic substances) [1]. In this study, we propose a hybrid biophotoelectrode that integrates intact microalgae cells with a WO_3 semiconductor electrode using polydopamine for cell entrapment and charge transfer enhancement. The biophotoelectrode delivers photocurrents of up to 24 μA cm^{-2} under visible light LED illumination with an incident light power below 6.0 mW cm^{-2}. The photoelectrode performance and the origin of electron flow are investigated, confirming a substantial contribution of immobilized microalgae to the overall photocurrent. In addition, we present a proof-of-concept application of the microalgae-based hybrid electrode in combination with a formate dehydrogenase biocathode for the implementation of a biophotoelectrochemical cell for the conversion of CO_2 to formate assisted by light. The system demonstrates the potential for coupling photosynthetic processes with bioelectrochemical conversion, achieving efficient and sustainable production of value-added chemicals. These findings advance our understanding of photosynthetic cell-electrode interactions in hybrid systems, offering insights for developing photobioelectrochemical devices and innovative conversion strategies for waste products.

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Cryo-EM of rationally designed photosystem I nanoassembly on graphene validates orientation-driven enhancement of photocatalytic performance

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Photosystem I (PSI)-based photovoltaic performance is hampered by short circuiting and charge recombination between randomly oriented PSI complexes on the electrode surface. Various types of the molecular interface have been designed to introduce control over order of PSI biophotocatalyst in the biomolecular nanodevices; yet, the precise orientation of the PSI complex has never been directly shown and quantified. Here, we report cryo-electron microscopy (cryo-EM) visualisation of PSI particles following domain-specific anchoring of this complex to the single layer graphene (SLG). By applying the rational design of the biomolecular interface, we directly demonstrate the preferential orientation of the PSI complex with its reducing side towards the SLG. This approach is effective to enhance anodic photocurrent generation 3-fold compared to the nanosystem with randomly deposited PSI particles. The cryo-EM visualisation of the PSI biomolecules on the graphene electrode surface provides the validation of the rational design of photocatalytic nanoarchitecture aimed at significantly improved photochemical performance.

Acknowledgements: This work was funded by the National Science Centre, Poland (Solar-Driven Chemistry 2 grant no. 2022/04/Y/ST4/00107). Part of this work was funded by the European Union (HORIZON-CL5-2022-D3-03 SUNGATE grant, Grant Agreement no. 101122061). MI gratefully acknowledges the financial support from the Polish National Agency for Academic Exchange (NAWA) (Bekker program, application number BPN/BEK/2022/2/00002). The Cryo-EM data were collected and processed at the Cryomicroscopy and Electron Diffraction Core Facility, Centre of New Technologies, University of Warsaw, Poland.

UVC light source to prevent airborne infection transmission

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Keywords: UVC, airborne infections, antimicrobial therapy

The pandemic outbreak and the antimicrobial resistance question have underscored the urgent need for innovative, effective strategies to mitigate airborne infections and overcome limitations of traditional preventive measures. Here we present *in vitro* and *in vivo* results of an innovative UVC light source acting as an invisible barrier against the spread of airborne viruses and bacteria. Although the well-known sterilizing properties of UVC, its use has always been limited to environmental applications due to mutagenic risks. Recently, UVC sources emitting at a wavelength $\lambda<230$ nm have attracted interest due to their very short penetration in tissue, which limits their interaction to the superficial stratum corneum (for the skin) or the first cornea layers for the eye. At the same time, RNA/DNA absorption for $\lambda < 230$ nm is still considerable to elicit photo-induced damage in the pathogen genetic material. This spectral range has thus been investigated in view of a possible application for environmental sterilization in the presence of humans.

Materials and Methods. Excimer KrCl lamps emitting 222-nm radiation were employed in a threefold experiment aimed at testing and validating the sterilization capacity of 222-nm light barriers against airborne infection. More than 30 relevant viruses and bacteria (wild-type reference strains and strains of clinical origin harbouring relevant antibiotic resistance mechanisms) were incubated and irradiated in both the liquid and the aerosol phase. Additionally, an *in vivo* experiment was conducted on mice, wherein the airflow was forced to move from a cage containing SARS-CoV-2 infected mice toward a second cage containing healthy ones, passing through a conduit equipped with a 222-nm light barrier, in controlled temperature and humidity conditions.

Results and Discussion. *In vitro* experiments showed a 3-log CFU reduction in CFU counts with light doses of 10-30 mJ/cm^2 for most bacterial strains tested (among which P. aeruginosa, S. aureus, and S. pneumoniae). S. pyogenes, H. influenzae, E. coli, and K. pneumoniae required a higher dose for the same efficacy. For viruses (among which SARS-CoV-2, Herpes Simplex, A/H1N1) more than 3-log reduction was achieved with 5-10 mJ/cm^2. Our approach was validated by in vivo experiments, where a 222-nm light barrier inside the conduit connecting the infected and healthy mice cages was able to completely prevent the healthy mice from contracting the infection.

Conclusions. The reported results point out a high antimicrobial efficacy of 222-nm light against all the tested viruses and bacteria strains. A 222-nm light barrier prototype was successfully tested to prevent in vivo airborne infections, suggesting a promising new approach in applications against human airborne infections.

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Photodynamic materials for the prevention and treatment of multidrug-resistant bacterial infections in humans and animals

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Keywords: Antimicrobial, Inactivation, Photodynamic, Quantum Dots

Efforts to control hospital acquired infections (HAIs) have been hampered by the emergence of drug-resistant pathogens, necessitating the pursuit of advanced functional materials that are capable of the self-disinfection of such microbes in hospital environments. To that end, we have explored antimicrobial photodynamic inactivation (aPDI) as an approach for pathogen reduction. *In vitro* aPDI studies were performed against bacteria and viruses employing photosensitizer-embedded or conjugated nanofibrillated cellulose, polyacrylonitrile or nylon nanofibers, dual-dyed wool/acrylic blended fibers, olefinic block copolymers, and spray coatings. As a recent example [1], we demonstrate that Cu-doped TiO_2 particles can successfully inactivate a wide range of pathogens with exposure to light for 90 min, including bacteria ranging from methicillin-resistant *S. aureus* (99.9999%, ~6 log units) to *K. pneumoniae* (99.93%, ~3.3 log units), and viruses including feline calicivirus (99.94%, ~3.4 log units) and HCoV-229E (99.996%, ~4.6 log units), with the particles demonstrating excellent robustness toward photobleaching. Furthermore, a spray-coated polymer film, loaded with the synthesized Cu-doped TiO_2 particles, achieved inactivation of methicillin-resistant *S. aureus* up to 99.998% (~4.8 log units). Building upon this work, as an alternative photosensitizer, we explored ligand-functionalized InP-based quantum dots (QDs) as an innovative class of nontoxic photosensitizer suitable for antimicrobial applications [2]. A hot injection method followed by functionalization via ligand exchange with 9-anthracene carboxylic acid (ACA) yielded the desired core/shell InP/ZnSe/ZnS QDs that were subsequently applied to cellulose via dip coating, with the resultant material exhibiting inactivation of MRSA (99.999%), as well as detection limit inactivation of human coronavirus 229E (HCoV-229E) and feline calicivirus (FCV). Finally, we will present more recent work [3] on a Rose Bengal (RB)-embedded poly(AEMA-TEGDMA) hydrogel as a novel photodynamic antimicrobial wound dressing for combating multidrug-resistant (MDR) infections. Fabricated via UV-induced photopolymerization, the hydrogel is composed of 2-aminoethyl methacrylate hydrochloride (AEMA) and tetraethylene glycol dimethacrylate (TEGDMA). Under portable LED illumination (400–700 nm, $\lambda_{max} = 445$ nm; 48 ± 2 mW/cm^2) for 15 and 30 minutes, the hydrogel demonstrated potent antimicrobial efficacy against both Gram-positive [methicillin-resistant *S. aureus* (MRSA; ATCC-44) and multidrug-resistant *Staphylococcus pseudintermedius* (MRSP)] and Gram-negative [multidrug-resistant *A. baumannii* (MDRAB; ATCC-1605) and NDM-1 positive *K. pneumoniae* (KP; ATCC-2146)] bacteria, routinely achieving up to 99.9999% (6-log) inactivation within 30 minutes. Antiviral studies against HCoV-229E, adenovirus, and rhinovirus also resulted in detection limit inactivation within 15 minutes.

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Rose Bengal photosensitizing effect on Candida albicans: new insights of an old molecule

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Keywords: antimicrobial photodynamic therapy; antifungal; Rose Bengal; light-induced death

Rose Bengal (RB) is an old photosensitizer, with the first results of its photodynamic effect against bacteria and yeasts dating from the late 70s, which already indicated its possible role as a singlet oxygen generator [1]. Since then, RB has been recognized as an efficient and safe photosensitizer, with the additional advantage of being soluble in water. Several formulations are already found in the literature, which increase RB stability and delivery rate against microorganisms of different types [2].

In this work we present a comparison of different cellulose particles loaded with RB (RB@CNC), showing that the covalent bond between the particles and RB defines the possible amount of RB charged into the particles, thus, defining their ROS rate production and their potential photoactivity. Furthermore, we explored the relation between fluence rate and concentration to achieve an effective antifungal disinfection of *Candida albicans* ATCC 10231, comparing the effect with free RB and revealing that the major contributor in this case is the RB loading rate.

Given that our prepared particles have sizes in the range of micrometres, it was unlikely that the nanoparticles would be able to penetrate inside the cells. Thus, we explored the mechanistic of the delivery of RB, using real-time monitoring with epifluorescence microscopy. We were able to observe that RB labels *C. albicans* cells when placed in proximity to the particles, in the presence or absence of a permeable barrier, with this effect not being time-related, as independent experiments show the stability of the nanoparticles in the range of days. The latest fact implies that the something in the environment of the particles and the cells favour the cleavage of the bonds, allowing RB to leak into the cells, with this additional factor being either the cells secretome or the ROS produced upon light irradiation.

Interestingly, this also showed a detail that seems to have been ignored for some years: RB is unable to penetrate into the cytoplasm of a healthy *C. albicans* cell, but irradiation and the cascade of ROS produced since the membrane or the periplasm of the cell are enough to trigger the cells death, which was marked by ROS production, RB permeabilization, a decrease on size of the cell and apparent organelles lysis, which together could indicate an apoptotic process when the death event was not immediate [3]. Then, although RB is a molecule that has been widely used as a photosensitizer for half a century, it still has secrets waiting to be revealed.

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Agricultural applications of Photodynamic Inactivation - 2025 update.

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Keywords: Photodynamic Inactivation, agriculture, plant pathogens, pest insects

Bacteria, fungi, and pest insects present significant challenges to growers, as they cause plant diseases or spoil of crops, thereby reducing yields. The use of pesticides, including antibiotics, in agriculture has a detrimental effect on the environment and contributes to the development of resistance. This presentation aims at providing a 2025 update on the application of Photodynamic Inactivation (PDI) in agriculture. Formulations of sodium magnesium chlorophyllin (Chl, food additive E140) are utilised as cost-effective and environmentally sustainable photoactive agents, and illumination is performed with either LED light (395 nm) or sunlight in the range of 26.6 to 300 J/cm^2. One hundred micromolar Chl induces a clear photoantimicrobial effect against the streptomycin-resistant bacterial phytopathogen *Erwinia amylovora* [1], and the fungus *Botrytis cinerea* is effectively controlled by PDI based on a formulation containing 224 µM Chl [2], even if resistant against conventional treatment. To fight the insect pest *Drosophila suzukii* Chl can be provided as food in 3% sucrose or sprayed onto insects. After feeding on 5 mM Chl (drug to light interval 9 h) and illumination with 78.9 J/cm^2 (LED 395 nm) 98.4% of all spotted wing *Drosophila* can be killed. Increased radiant exposure (LED 395 nm, 315.6 J/cm^2) allows for usage of lower Chl concentrations (1 mM, 97.5% kill). If using sunlight (294.5 J/cm^2) for Chl (5 mM) activation all flies are erased two days after illumination. Fluorescence microscopy confirms Chl accumulation in the flies' intestines [3]. Formulations of Chl can also be employed for postharvest photodecontamination of fruits, as proven by using an orange peel model and *Penicillium digitatum* as fungal model organism [4]. In conclusion, PDI based on formulations of Chl is applicable in agriculture to combat bacterial and fungal plant diseases and pest insects.

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Acknowledgements:
The authors are grateful to Jun Liu PhD, Corteva Agriscience, Indianapolis, IN, USA and to Michael Fefer PhD, Whitby Ag Consulting, Whitby, ON, Canada for providing their expertise. The study was supported by Suncor AgroScience, Mississauga, ON, Canada and by Nutrien Ag Solutions, Calgary, AB, Canada.

Sustainable Synthesis and Photodynamic Antifungal Activity of a Chitosan-Riboflavin Conjugate for Agricultural Applications

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Keywords: Mechanochemical synthesis, chitosan-riboflavin conjugate, photodynamic activity, sustainable biofungicide

Agricultural production is crucial for global food security, yet the control of phytopathogenic fungi such as *Botrytis cinerea* and *Penicillium digitatum* remains a major challenge. The widespread use of synthetic fungicides is increasingly restricted due to environmental and health concerns, emphasizing the need for sustainable alternatives. In this study, we investigated the photophysical and photochemical properties of a chitosan-riboflavin conjugate (CH-RF) as a light-activated biofungicide.

A key aspect of this work was the replacement of pyridine as a solvent in the acylation of riboflavin with a mechanochemical approach, reducing the environmental impact while maintaining reaction efficiency. The resulting CH-RF conjugate exhibited significant antifungal activity under light irradiation. In vitro and in vivo assays demonstrated its efficacy against *P. digitatum* [1] and *B. cinerea* [2], inhibiting fungal growth and reducing disease severity in fruit models. Notably, CH-RF outperformed commercial biofungicides in postharvest grape and citrus preservation.

These results highlight the potential of CH-RF as an eco-friendly, photodynamically active biofungicide. Its light-driven antifungal mechanism, combined with a sustainable synthetic approach, aligns with green chemistry principles and presents a promising alternative for agricultural applications.

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Acknowledgements: APQ thanks SACRU for a Research Mobility fellowship

Sunlight-Powered Fungicide Conjugates: Fighting Fungal Resistance

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Keywords: pest resistance, fungicides, photodynamic effect, photodynamic conjugates, azoxystrobin.

Through our work, we want to remind the scientific and industrial communities that photodynamic pest control is an effective, yet forgotten approach, which carries interesting advantages, such as being effective against resistant organisms, a major problem of many synthetic pesticides. In the 1970s and 80s there was a considerable academic interest in the use of photosensitizers in crop protection. Many field studies showed that many different sensitizers were effective pesticides against many crop-damaging organisms, such as fungi, bacteria or insects, leaving the plant largely undamaged. As photodynamic pest control became a popular topic, a large part of the Agrochemicals section of the 1986 and 1995 ACS symposia was devoted to such photodynamic pesticides. Since that time, however, the topic has lost relevance in the scientific and industrial community. This is largely due to the highly competitive nature of the pesticide market at the time, with highly effective and selective synthetic pesticides being produced at cheap costs. This picture has changed in recent years. The use of synthetic pesticides in recent decades has come at a high environmental cost due to bioaccumulation problems, non-target toxicity and pest resistance. For these reasons, the agricultural market is being depleted of effective crop protection products at an alarming rate. Photodynamic compounds could be extremely useful in some scenarios where standard pesticides are ineffective, or if applied with modern precision application techniques that lower their risk profile. In our work, we present a novel design of photodynamic fungicides, which consists in the covalent binding of a photodynamic sensitiser to the molecular structure of a commercial fungicide. In our examples, we observed a synergistic effect between the two expected biocidal modes of action of these conjugates: the biochemical toxicity, intrinsic to the fungicide structure, and the photodynamic effect under light. In this way, we upgraded classical fungicides, such as azoxystrobin, by giving it a new mode of action while retaining their biochemical toxicity. We also demonstrated that such conjugates were able to kill under light two resistant strains of Botrytis cinerea, a fungus affecting strawberry plants and wine grapes, which were otherwise immune to azoxystrobin.

Taming the Swamp Thing: chlorophyllin-based Photodynamic Inactivation against Candida auris dynamic biofilm

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Keywords: Photodynamic Inactivation, *Candida auris*, Dynamic biofilm, One Health, chlorophyllin

Based on a theory, the yeast *Candida auris* arose from Indian wetlands through climate change and human input to be a nosocomial threat and thus is a perfect example for the relevance of the One Health concept [1]. Existing in these harsh conditions, *C. auris* gained thermo- and halotolerance and relocated to human habitats, where it is believed to have picked up resistance genes to common antifungal agents. The colonization of healthy human skin brought the yeast into hospitals, where it became resistant to antiseptics, selecting it to be a massive threat to fragile patients [2]. *Candida auris* is extremely persistent on all kinds of surfaces, as it can grow into a robust biofilm, which enables repeated reinfection or colonialization of patients [3]. In this study, we established for the first time a dynamic biofilm protocol for a *C. auris* type strain and subjected both, suspension cultures and the biofilm to Photodynamic Inactivation. As photosensitizers the biocompatible and economic sodium-magnesium-chlorophyllin and the semi-synthetic chlorin e6 derivative with cationic moieties B17-0024 were used. After a drug to light interval of 15 min, samples were illuminated using blue (395 nm, 25 J cm^{-2}) or red (600 – 700 nm, 100 J cm^{-2}) light. The antimicrobial effect of a 99.9% reduction of *C. auris* was exceeded with 50 µM Chl and B17–0024 plus red light and with 10 µM B17–0024 plus blue light. Complete eradication of the *C. auris* dynamic biofilm was achieved if phototreated with 100 µM Chl and blue light or 100 µM B17–0024 and red light. These results indicate that Photodynamic Inactivation using chlorophyllin based photosensitizers may serve as a simple, fast, and effective approach to disinfect surfaces from newly emerging pathogens, even if resistant to conventional treatments.

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Acknowledgements:

The authors are grateful to Suncor AgroScience, Canada and to Nutrien Ag Solutions, Canada for their support.

Antimicrobial Photodynamic Inactivation: A Promising Approach to Prevent Bacterial Infections in Aquaculture Systems

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Keywords: *Vibrio anguillarum*, *Artemia franciscana*, *Psetta maxima*, potassium iodide

Vibrio anguillarum is a pathogenic bacterium responsible for high mortality rates and severe financial losses in the aquaculture industry [1]. This bacterium is often found in *Artemia* nauplii (brine shrimp), a common fish live feed, making it a primary vector for *V. anguillarum* transmission to farmed fish [2]. The aim of this study was to assess the potential of antimicrobial Photodynamic Inactivation (PDI) for preventing *V. anguillarum* infections in aquaculture. To this purpose, the efficiency of PDI was evaluated in: i) photoinactivating *V. anguillarum* in seawater; ii) preventing infections in turbot (*Psetta maxima*) previously challenged with *V. anguillarum*; and iii) decontaminating *Artemia franciscana* nauplii contaminated with *V. anguillarum.* These assays were performed using the tetracationic porphyrin 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin (TMPyP; 5.0 µM), combined with the well-known PDI adjuvant potassium iodide (KI, 10-100 mM), under white light irradiation (400-800 nm, 100 mW.cm^{-2}). The results showed that PDI mediated by TMPyP + KI was efficient to reduce *V. anguillarum* concentration in seawater to the detection limit of the method (7 log CFU mL^{-1}) in less than 10 min. Additionally, preliminary toxicity assays identified a non-toxic concentration for the *in vivo* studies on *P. maxima* and brine shrimp: TMPyP (5.0 µM) + KI (10 mM). This combination significantly reduced the *V. anguillarum* concentration in *A. franciscana* nauplii by over 3 log CFU mL^{-1} after 30 min of irradiation. Furthermore, in the *P. maxima* experiments, the application of this treatment for only 5 min prevented severe symptoms but did not reduce fish mortality.

In this communication, a detailed discussion of these findings will be presented, showing that PDI can be an effective approach for preventing *V. anguillarum* infections in aquaculture by disinfecting water and fish live feed; however, further studies are required to address fish mortality once the infection process has begun.

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Acknowledgments: The authors thank the University of Aveiro and FCT/MCTES for financial support to the UID Centro de Estudos do Ambiente e Mar (CESAM) + LA/P/0094/2020 the UID/50006 LAQV-REQUIMTE—Laboratório Associado para a Química Verde—Tecnologias e Processos Limpos, and to project PREVINE—FCT-PTDC/ASP-PES/29576/2017), through national funds (OE) and where applicable co-financed by the FEDER-Operational Thematic Program for Competitiveness and Internationalization-COMPETE 2020, within the PT2020 Partnership Agreement. Cátia Vieira (C.V.) thank FCT for her Ph.D grant (SFRH/BD/150358/2019).

Cell viability, ROS production, lipid peroxidation and membrane damage of L. monocytogenes after UVA and Blue Light treatment

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Keywords: Blue Light; UVA; food pathogens; microbial damage.

Blue Light (BL) has recently gained attention for its potential as a non-thermal, chemical-free antimicrobial strategy. Similar to near-ultraviolet A (UVA), its inactivation mechanism involves the intracellular generation of reactive oxygen species (ROS), leading to microbial damage.
This study aimed to explore the effect of two close wavelengths, 365 nm (UVA) and 405 nm (BL) to understand a possible relationship between microbial inactivation and ROS production and lipid peroxidation and membrane damage of *L. monocytogenes*, a dangerous food pathogen [1], [2], [3].
L. monocytogenes samples were exposed to both wavelengths and analyzed using cell viability assays, DCFH-DA fluorescence (for ROS quantification), TBARS assay (for lipid peroxidation), and Real-Time PCR with PMAxx (to assess membrane damage).
Both wavelengths achieved a similar microbial reduction (~2-log CFU), accompanied by substantial ROS production (~14,000 RFU). ROS levels were slightly lower under 365 nm exposure.
Interestingly, lipid peroxidation showed an opposite trend: UVA (365 nm) induced significantly higher levels (~2 nmol/well) compared to 405 nm (~1 nmol/well), suggesting differential oxidative damage at the membrane level. Real-Time PCR with PMAxx revealed discrepancies with traditional viable cell counts, possibly indicating the presence of viable but non-culturable (VBNC) cells or wavelength-specific differences in oxidative stress responses.
These findings provide insights into the photoinactivation mechanisms of UV and BL technologies and support their potential application as eco-sustainable antimicrobial interventions in the food industry [4].

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Photodynamic Decontamination of food: assessing surface challenges against Listeria monocytogenes

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Keywords: Photodynamic Inactivation, food safety, *Listeria monocytogenes*, chlorophyllin

Listeria monocytogenes is a foodborne bacterium of concern. It commonly leads to self-resolving gastroenteritis, nevertheless, it presents a high risk to immunocompromised individuals and, in the case of pregnancy, can cause miscarriage [1]. Numerous outbreaks have been reported through ready-to-eat foods [2]. Although heat treatment is widely used for food decontamination, it is unsuitable for fresh produce such as fruits and vegetables. Other physical (e.g., UV, gamma irradiation) and chemical (e.g., ozone) methods may alter organoleptic properties or face limited consumer acceptance [3]. Photodynamic Inactivation of microorganisms (PDI) represents a promising alternative, especially if based on natural photosensitizers [4]. In this study we assess the effectiveness of PDI *in situ* using three food model systems with different surface properties: apples (smooth surface), strawberries (uneven surface), and kiwis (fuzzy and hairy surface). Additionally, the impact of contamination sequence on decontamination efficacy was evaluated. Sodium-magnesium-chlorophyllin (Na-Mg-Chl) is used as photosensitizer since it is approved as food additive E140 [5]. Fruits were cut into 1 cm² squares and inoculated with *L. monocytogenes*. A 100 µM Na-Mg-Chl solution was applied either before or after bacterial inoculation. All samples were then illuminated using a 395 nm LED array at a radiant exposure of 15 J/cm². When *L. monocytogenes* was applied to various fruit surfaces via sequential deposition, first of the bacteria followed by Na-Mg-Chl coating, a 5.96-log reduction was observed on apples, a 5.71-log reduction on strawberries, and a 6.02-log reduction on kiwis. When Na-Mg-Chl coating was applied prior to bacterial deposition decontamination rates were very comparable, ranging from 5.61-log to 6.74-log. These findings demonstrate that PDI is a highly effective approach for decontaminating a variety of fruit surfaces, regardless of the sequence of contamination, by this reducing public health risks and economic losses in the food industry while maintaining consumer trust.

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Acknowledgements:
The authors are grateful to Suncor AgroScience, Mississauga, ON, Canada and to Nutrien Ag Solutions, Calgary, AB, Canada for their support.

Photopheresis: 40 years on

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Keywords: photochemotherapy, 8-methoxyprsoralen, dermatology

Photopheresis (extracorporeal photochemotherapy) was invented more than 40 years ago to expose leukocytes from the peripheral blood of patients with leukemic variants of cutaneous T-cell lymphomas (Sézary syndrome) to photochemotherapy with 8-methoxypsoralen. Special equipment was developed and refined to isolate leukocytes from the peripheral blood, treat the isolate with photoactivated 8-methoxypsoralen and re-infuse it to the patients. The concept behind this treatment was the hypothesis of T cell immunization, i.e. the experimental observation that exposure of the immune system to physically altered T cells can cause specific inhibition of T cell-mediated autoimmunity. Since its introduction, photopheresis has been tried in a variety of diseases and has been shown to be clinically effective, in cutaneous T-cell lymphoma, graft-versus-host disease, systemic sclerosis and solid organ transplant rejection. Although the mechanisms of action are not yet fully understood, it appears that changes in antigen presentation and the generation of regulatory T cells may be related to the observed clinical effects. After more than 40 years of clinical and experimental research, photopheresis has found its place in clinical practice, alone or in combination with other therapies, as a safe and effective option for many patients. Further research will help to refine the clinical indications and fill the gaps that still exist in our knowledge of when, how and why photopheresis works.

Phototherapy of Mycosis fungoides

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Keywords: phototherapy; Mycosis fungoides; UVB; PUVA;

Cutaneous T-cell lymphomas (CTCLs) are a group of rare non-Hodgkin lymphomas (NHLs) characterized by proliferation of malignant T-lymphocytes clones in the skin.

Mycosis fungoides (MF) is the most prevalent form of CTCL, characterized by a chronic disease course, clinically presenting with erythematous patches and plaques in early stages, potentially progressing to cutaneous tumors and involvement of other organs. In early stages, skin-directed therapies (SDT) are primarily employed to provide effective palliative care. Treatments like phototherapy with ultraviolet A (PUVA) in combination with 8-methoxypsoralen, or ultraviolet B (UVB) radiation, have proven efficacy in inducing remissions. Phototherapy can be safely combined with systemic agents, especially interferon-alpha and retinoids. Updated treatment guidelines now offer evidence-based algorithms for stage-specific treatment. PUVA and narrow-band UVB (NB-UVB) are recommended as first-line therapies for early-stage MF, while combination treatments are reserved for advanced or refractory cases. Lack of evidence remains regarding the optimal dosing, maintenance strategies, and the role of newer phototherapies (e.g., ultraviolet A1 radiation, excimer lasers, photodynamic therapy) in treating MF.

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Modification of extracorporeal photopheresis with 5-aminolevulinic acid

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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.

Identification of clones by TCR Vβ repertoire analysis supports diagnosis of leukemic cutaneous T-cell lymphoma

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Keywords: cutaneous T-cell lymphoma, Sézary syndrome, TCR Vβ repertoire analysis

Erythrodermic leukemic cutaneous T-cell lymphoma (L-CTCL) comprises progressive erythrodermic mycosis fungoides (MF) with infiltration of blood and Sézary syndrome (SS). Clinically established diagnostic procedures do not always allow sufficient distinction of L-CTCL and erythrodermic conditions of other origin. Diagnosis of L-CTCL might be supported by identification of clones via TCR Vβ repertoire analysis.

In the time span between March 2017 and April 2024 blood samples were collected from 63 patients with erythroderma (female/male: 22%/78%; mean age: 64±14 years). Patients included had a diagnosis of L-CTCL in 17 cases (7 patients with SS, 10 patients with erythrodermic leukemic MF), whereas 46 patients presented with erythroderma due to another origin (23 patients with non-leukemic MF, 13 patients with atopic dermatitis, 10 patients with other conditions). Whole peripheral blood mononuclear cells (PBMCs) were isolated from blood samples and examined for T-cell clones by applying a multiparametric analysis tool designed for quantitative determination of the TCR Vβ repertoire of T-lymphocytes via flow cytometry (Beckman Coulter #PN IM3497, USA).

As significant T-cell clones were detected in blood samples of all 17 patients with diagnosed L-CTCL, TCR Vβ repertoire analysis showed a 100% sensitivity in identification of L-CTCL and thereby presented superior to FACS with conventional T-cell panels (sensitivity of 70.6% [12 of 17 patients]). Intriguingly, T-cell clones were also found in 5 of 23 patients (21.7%) with non-leukemic MF and 5 of 23 patients (21.7%) with benign erythrodermic conditions such as atopic dermatitis (n=3), GvHD (n=1) and psoriasis (n=1), indicating a 78.3% L-CTCL specificity of the method ($p<0.0001$). A further significant difference between L-CTCL and no-L-CTCL group could be revealed, when comparing percentages of clonal TCR-Vβ repertoire (median±IQR in patients with L-CTCL (n=17): 68.4%±25.9%; median±IQR in patients with other conditions (n=10): 43.3%±23.6%; [$p<0.0001$]).

In summary, TCR Vβ repertoire analysis represents a highly sensitive method regarding detection of malignant clones in the blood of patients with L-CTCL. Moreover, the methodology might support therapeutic decisions, as it allows screening for molecules such as CCR4, CD30, CTLA-4 and CD52.

Wavelength-Dependent Modulation of Inflammatory Cytokines by Photobiomodulation in Cutaneous Wound Healing

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Keywords: photobiomodulation, laser, inflammation, wound healing.

Introduction: Wound healing is a complex process involving coordinated inflammatory and reparative phases. Photobiomodulation (PBM) enhances tissue repair by modulating cell activity and cytokine expression [1], and its effectiveness is highly dependent on parameters such as wavelength and energy dose. Thus, this study evaluated the effects of different laser wavelengths (red, green, blue, violet) on TNF-α, IL-6, IL-1β, and IL-10 expression in a rat wound model.

Materials and Methods: Wistar rats (n = 12/group) received two excisional wounds on the dorsal cervical region. Only the cranial wound was treated daily with PBM for five days using custom diode lasers (9 J/cm^2): red (660 nm), green (532 nm), blue (470 nm) and violet (405 nm). The caudal wound served as internal control. A custom tip prevented light dispersion. A separate control group received no PBM. Samples were collected on days 3, 7, and 14 for ELISA-based quantification of cytokines. Data were analyzed by two-way ANOVA with Bonferroni post hoc test ($\alpha = 0.05$).

Results and Discussion: PBM altered cytokine expression in a wavelength- and time-dependent manner. IL-1β levels were higher in the non-irradiated control group at day 3 compared to all PBM-treated groups ($p < 0.05$), with undetectable levels in the red group by day 14. IL-6 was lower in the red group than in the blue and violet groups at day 3 ($p < 0.05$), while blue light induced sustained IL-6 elevation across all time points. IL-10 increased in all PBM groups, especially red and green, with RC and GC groups- representing non-irradiated wounds- showing the highest levels at all time points ($p < 0.05$), suggesting distant effects. TNF-α levels did not differ significantly among groups ($p > 0.05$), but were undetectable or near zero in the red, blue, and violet groups by days 7 and 14. This reduction may support healing, as reported in previous LED-based studies [2,3]. Our findings demonstrate that red and green PBM promoted a favorable anti-inflammatory profile, characterized by early suppression of IL-1β and IL-6 and sustained upregulation of IL-10. In contrast, blue light appeared to prolong the inflammatory phase. These results highlight the therapeutic potential of red and green laser wavelengths, applied at a dosimetry of 9 J/cm², in modulating inflammation and enhancing tissue repair.

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Photocaged Small Molecule Inhibitor of CD73: A Strategy for Light-Guided Cancer Immunotherapy with Reduced Off-Target Effects

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Keywords: Photoprotection group; Tumor microenviroment; Immunetherapy; Cancer;

While therapeutic blockade of the PD-1/PD-L1 immune checkpoint has shown promise, only a limited subset of patients with advanced malignancies benefit from this approach. Many solid tumors evade immune detection through alternative, non-overlapping immunosuppressive mechanisms. Among these, the CD73–adenosine pathway has emerged as a key immunosuppressive axis within the tumor microenvironment. CD73 catalyzes the stepwise dephosphorylation of pro-inflammatory extracellular ATP—released by cancer cells—into immunosuppressive adenosine, one of the most potent modulators of immune suppression.[1] However, current CD73 inhibitors suffer from a lack of tumor selectivity, and systemic inhibition may trigger serious immune-related adverse effects.

To overcome this challenge, we introduce a photoprotective group (PPG) strategy to achieve light-controlled activation of small-molecule inhibitors.[2] Here, we report the development of a green-light-activated, photocaged CD73 inhibitor that enables precise spatial and temporal regulation of its immunosuppressive activity. The caged compound remains inactive and non-toxic in the absence of light, offering a promising strategy for localized, light-triggered immune checkpoint inhibition. This study presents a proof-of-concept for achieving precise spatial and temporal control of CD73 blockade, with the potential to minimize off-tumor toxicity and enhance the therapeutic window of immune checkpoint inhibitors.

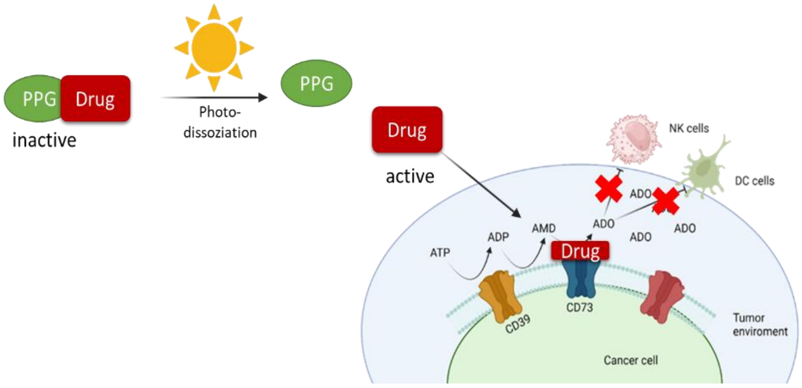

Figure 1. Light induced activation of CD73 receptor

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Development of novel near-infrared photosensitizers to induce immunogenic cell death for deep-tissue cancer therapy

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Keywords: Photodynamic therapy, photosensitizers, near-infrared, apoptosis, immunogenic cell death

Introduction: The induction of immunogenic cell death (ICD) has emerged as a promising strategy in cancer immunotherapy. ICD is a regulated form of cell death that not only eliminates the tumor cells but also activates an anti-tumor immune response and establishes enduring immunological memory. One effective approach for triggering ICD is the use of photodynamic therapy (PDT). PDT employs light-activated photosensitizers (PSs) to generate reactive oxygen species, causing oxidative damage and tumor cell death. The oxidative stress and cellular damage induced by PDT can initiate ICD through the release of damage-associated molecular patterns (DAMPs) (adjuvanticity) and the exposure of tumor antigens (antigenicity). While conventional PDT has shown potential, its clinical application remains limited due to the superficial tissue penetration depth of commonly used PSs, which are typically activated by light in the 600-700nm range. This restricts treatment to easily accessible tumors. To overcome this limitation and expand the therapeutic capacity of PDT, PSs are developed that can be activated by near-infrared (NIR) light, which offers a deeper tissue penetration and enables the activation of PSs in larger and more deeply located tumors. Hence, the objective of this project is to evaluate the anti-cancer and immunogenic properties of novel NIR sensitive compounds, provided by Agfa-Gevaert NV (located in Mortsel, Belgium), with the aim of advancing PDT-based strategies for deep-tissue cancer treatment through the induction of ICD. **Materials and Methods:** In this study, we performed a screening of 92 NIR-activated PS molecules and identified one promising molecule for PDT-induced ICD. The absorbance spectra of the PSs were measured with a multimode microplate reader and the light-induced cytotoxicity following irradiation at 780nm was assessed using the MTS assay and flow cytometry. In addition, cellular uptake of the PSs and (sub)cellular localization were analyzed by confocal microscopy. Lastly, exposure of phosphatidylserine, which is a phospholipid that translocated to the outer leaflet of the cell membrane in early cell death stages and serves as an 'eat-me' signal, was measured as it is commonly associated with regulated (and immunogenic) forms of cell death, such as apoptosis. **Results:** The results demonstrate that out of the 92 screened PS molecules, only one compound, called C86, was able to induce significant cell death upon NIR-light irradiation and exhibited minimal dark toxicity (*i.e.,* when not exposed to NIR light). Significant cell death induction by C86 was confirmed across multiple murine cancer cell lines, including MCA205 fibrosarcoma, CT26 colon carcinoma, B16 melanoma and GL261 glioma cells. Moreover, confocal images revealed accumulation of C86 in the cytoplasm of the cells, and, upon light irradiation, the presence of apoptotic blebbing was observed. Furthermore, analysis of phosphatidylserine externalization showed an increase in exposure in approximately 10% of the cells, indicating apoptosis, while the majority of dying cells exhibited features of necrotic cell death modalities. These results suggest that C86 induces a combination of different cell death pathways, potentially with immunogenic properties. Further research will focus on characterizing the induced cell death modalities and investigating the potential of C86 to induce ICD and anti-tumor immune responses. **Conclusion:** In conclusion, we identified C86 as a promising NIR-activated PS capable of inducing significant cancer cell death. The observed combination of apoptotic and necrotic features suggests the potential for anti-tumor immunogenic effects.

Multifaceted Effects of a Multiple Nitric Oxide Photoreleaser and its Photoproducts on Amyloid-β Aggregation

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Keywords: Light, Nitric Oxide, Alzheimer, Organofluoride

The pathological aggregation of β-amyloid (Aβ) peptides into insoluble fibrils is a defining hallmark of Alzheimer's disease (AD).[1] Among the post-translational modifications that influence Aβ behavior, nitration and nitrosation mediated by nitric oxide (NO) derivatives play a crucial yet not fully elucidated role in modulating both aggregation and toxicity.[2,3] This study explores the effects of a nitric oxide photodonor (NOPD) that sequentially releases two NO molecules under visible blue light irradiation. A marked inhibition of $A\beta_{1-40}$ aggregation is observed when a substantial amount of NO (approximately 120 µM) is photoreleased during the initial phases of the aggregation process. Conversely, a comparable release of NO over prolonged periods fails to exert the same effect. Intriguingly, the NOPD kept in the dark promotes aggregation, underscoring the importance of light-controlled activation. The stable organofluorinated photoproducts generated along with the release of the first and second NO molecules also exhibit inhibitory effects on $A\beta_{1-40}$ aggregation, both when tested individually and in combination. Complementary molecular dynamics simulations provide further insight into the binding interactions of the NOPD and its photoproducts with key residues of the $A\beta_{1-40}$ peptide.

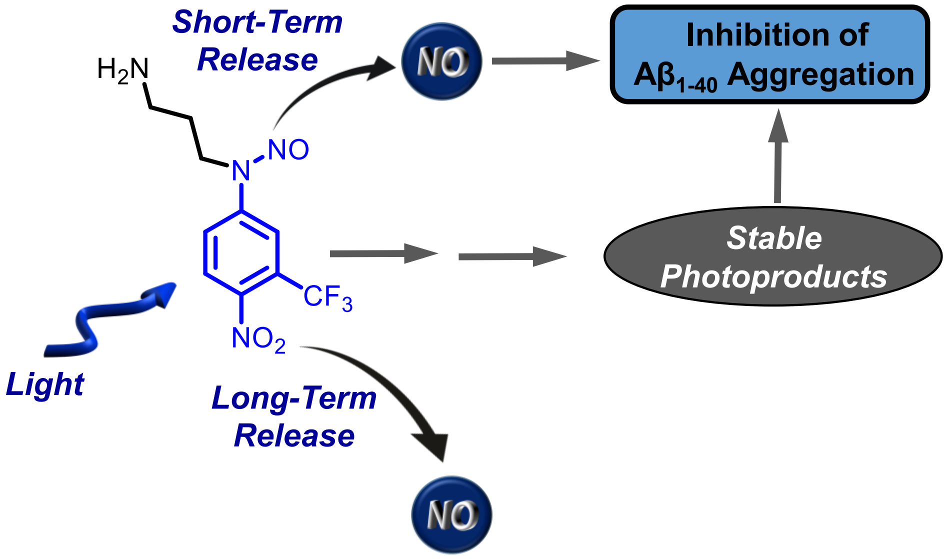

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Photo-antibacterial efficiency of metalloporphyrins bearing triphenylphosphonium units

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Keywords: Metalloporphyrin; Triphenylphosphonium; Photosensitizer; Bacteria

Antimicrobial therapy is widely used in modern medicine and has contributed to longer lifespans [1]. However, its increasing and often indiscriminate use, along with rising microbial resistance, highlights the need for alternative approaches [2].

Tetrapyrrolic macrocycles, including porphyrins, have garnered significant interest from the scientific community owing to their distinct characteristics, rendering them one of the most extensively researched compound families. Their suitability for antimicrobial photodynamic therapy (aPDT) is underscored by several specific properties. These include their absorption features within the visible region of the electromagnetic spectrum, their capability in generating reactive oxygen species (ROS), minimal cytotoxicity in the absence of light, stability, and compatibility with biological systems [3,4]. In particular, metalloporphyrin derivatives exhibit enhanced photodynamic efficacy compared to their corresponding free-base derivatives. This improvement is often attributed to their superior ROS production [5].

In this context, the main objective of this work was to evaluate the influence of metal ions on the photochemical and photophysical properties of three cationic porphyrin-triphenylphosphonium conjugates. In this communication will be discussed these properties of a series of metalloporphyrins prepared through the coordination of the metal ions Zn(II), Co(II) and Pd(II) within the porphyrin-triphenylphosphonium core and assessed their photosensitizer efficacy against Gram-positive methicillin-resistant *S. aureus* (MRSA) and Gram-negative *Escherichia coli*.

Funding/Acknowledgements: This research work received financial support from the University of Aveiro and FCT/MCTES to support UID/50006 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos research unit. NMM Moura gratefully acknowledges FCT for an Assistant Research Position (2023.06495.CEECIND).

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Engineering Hypoxia-Tolerant Photosensitizers: An Iridium–Phthalocyanine Approach

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Keywords: iridium(III) photosensitizers; zinc phthalocyanines; photodynamic therapy; hypoxia.

Despite the increasing interest in photodynamic therapy (PDT) as a minimally invasive cancer treatment, its clinical efficacy remains limited by two persistent challenges: the lack of photosensitizers that absorb in the red/near-infrared (NIR) range [1], and the reduced effectiveness of ROS generation in hypoxic tumor environments [2].

In this work, we present a novel Ir(III)-phthalocyanine conjugate (Ir-ZnPc), the first of its kind, engineered to overcome both limitations through a high-efficiency synergistic PDT treatment [3]. By integrating the optical advantages of the phthalocyanine core with the potent phototoxicity of cyclometalated Ir(III) complexes, and encapsulating the conjugate into smart redox-responsive nanocapsules, we achieve a platform with enhanced solubility, cellular uptake, and therapeutic precision.

Our results show promising behavior under conditions that typically hinder PDT performance—including oxygen-deprived 3D tumor models. The unique photochemical features and biological effects of this system will be discussed, revealing a new strategy to unlock the full potential of PDT in challenging tumor microenvironments.

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Potential of a radioluminescent light source for photobiomodulation in the treatment of Parkinson's disease

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Keywords: Parkinson's disease; photobiomodulation; radioluminescence; aerogel

Photobiomodulation is a promising technique aimed at slowing down neurodegeneration by illuminating specific brain regions depending on the neurodegenerative disease. Clinatec is currently conducting the first clinical trial with 7 Parkinson's disease patients implanted with an intracerebral optical fiber emitting light at 670-nm wavelength [1].
In this context, a RadioLuminescent (RL) device based on luminescent materials excited by the tritium beta-decay is of high interest. This approach could be less invasive, may offer more efficient light delivery to tissue due to its compactness and diffuse illumination, and extended energy autonomy, all contributing to enhanced patient comfort and treatment efficacy.
Several strategies for coupling tritium with luminescent materials are documented, including the use of tritium gas, tritiated water or a solid tritiated matrix [2].
In this presentation, we will show quantitative results on the development of a millimeter-sized RL device in which tritium beta-decay excites luminescent materials. The adopted strategy is based on an organic matrix serving as both tritium and luminescent material reservoir. Cylindrical samples made of either silica or cellulose aerogels have been fabricated. For light emission, red-emitting phosphors and quantum dots such as Y_2O_3:Eu^{3+}, YVO_4:Eu^{3+}, $CuInS_2$ and $AgInS_2$ have been selected and their optical properties characterized using cathodoluminescence, synchrotron X-ray tomography and BET surface area analysis. Samples were placed within an integrating sphere and exposed to tritium gas atmosphere (from 0.2 to 1.2 bar), allowing us to quantify their optical performance while varying the tritium pressure. Our findings suggest a direct dependence between the radiance and tritium pressure as well as a potential mechanism for trapping tritium atoms in micrometer-sized luminescent materials. On top of that, we could correlate the brightness to the concentration and material composition.
The aerogel showing the best optical performance has been exposed to tritium gas and then sealed in a borosilicate capsule containing 0.6 bar of tritium gas. This RL capsule is now under study to evaluate its long-term aging that will be shown here.
This ongoing work has led to a scientific publication [3] and it holds promise for self-powered luminescent devices, potentially optimizing photobiomodulation in clinical applications.

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Ca^{2+} signals interplay with ROS in Br-SQ-C4 induced phototoxicity

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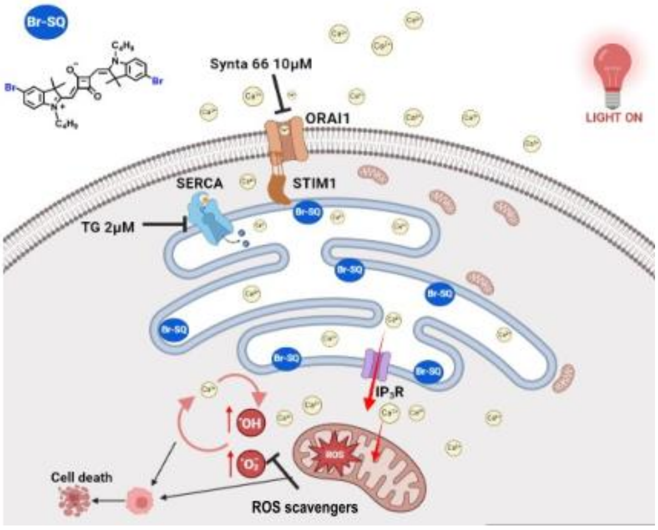
Keywords: PDT; PDAC; ROS; Ca^{2+} signals

Photodynamic Therapy (PDT) is based on the administration of a photosensitizer (PS) which accumulates into tumor cells and activated using specific light sources. This result into the production of ROS and singlet oxygen ($^1O_2$), responsible for cell death [1]. Since it is well known in literature the interplay between ROS and Ca^{2+}[2], we are wondering about the ability of PS to induce the production of Ca^{2+} signals upon light activation. Since our laboratory has already worked on the synthesis and characterization of Polymethine Dyes (PMD) applied in the PDT field [3], [4], in the present work we proposed Br-SQ-C4 [5] as a promising PS. This absorbs light at 650nm, and for this reason, can be applied for the treatment of tumors with dense stroma, such as PDAC (Pancreatic Ductal AdenoCarcinoma). This is also characterized by a strongly acidic core, which selects aggressive and chemotherapy resistant cell clones, as we already demonstrated [6], [7].

In this work we used PDAC cell lines: PANC-1 CT (maintained at pH_e 7.4), and PANC-1 pH selected (maintained for 1 month at pH_e 6.6, mimicking the internal tumor core) [6], to observe the intracellular dynamics, using real time imaging with fluorescent probes for ROS and Ca^{2+} signals. In particular, we demonstrated that the PANC-1 pH selected are more sensitive than PANC-1 CT to Br-SQ-C4 phototoxicity, which produces ROS ($O_2^{\bullet -}$) and Ca^{2+} signals upon irradiation using RED-LEDs (8 minutes of irradiation, λ=640 nm; Fluency: 3.84 J/cm^2) in both the cell lines even if with different dynamics. Moreover, we are working to demonstrate the existence of an interplay between these signals, as we already did in MCF-7 cell lines (adenocarcinoma of human mammary glands) (work in submission).

Since some Ca^{2+} channels display a ROS-dependent regulation, we wonder if any of these could be involved in Br-SQ-C4 phototoxicity, which would be a further validation of their interplay. We took into consideration the role of Orai1, involved in the SOCE (Store Operated Ca^{2+} Entry), which opens when the endoplasmic reticulum (ER) Ca^{2+} levels drop. Since Br-SQ-C4 localizes mainly in the ER we are particularly interested in this mechanism. Using Synta66, specific inhibitor of Orai1, in combination with Br-SQ-C4, we are assessing the cell viability, ROS and Ca^{2+} dynamics of both PANC-1 CT, PANC-1 pH selected and MCF-7, to better understand the intracellular signal transduction, which can be crucial to find a possible target for the enhancement of Br-SQ-C4 phototoxicity.



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A novel ruthenium-based PACT treatment for uveal melanoma: Formulation advancements and preclinical insights

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Keywords: PACT, Uveal melanoma, cancer treatment

Background and Objective. Uveal melanoma (UM) is the most common primary intraocular cancer in adults, with a global incidence of approximately 4.3 cases per million[1]. Despite effective primary treatments, UM often leads to significant vision loss, and liver metastasis occurs in about half of the patients[2]. The PACT4EYE project focuses on the development of photoactivated chemotherapy (PACT) using a novel ruthenium-based prodrug (Ru-MTI)[3], which is activated by light to release the cancer-targeting agent MTI (Microtubule polymerization Inhibitor). Unlike traditional therapies that rely on oxygen for activation, Ru-MTI is effective even in hypoxic tumor environments, offering a distinct advantage for treating resistant tumors. This study focuses on developing Ru-MTI formulations to enhance solubility, stability, reduce aggregation, and improve cellular uptake, facilitating clinical application.

Materials and Methods. As part of the PACT4EYE project, a primary screening was performed on the zebrafish model to identify responsive tumors. Selected patient-derived xenograft (PDX) models were engrafted in mice and tested for efficacy and toxicity under various conditions. The formulation of Ru-MTI was optimized to enhance solubility, prevent aggregation, and increase stability for intravenous administration. The caged Ru-MTI prodrug is soluble in water-based solutions, unlike the uncaged inhibitor, which is insoluble. This solubility enables intravenous injection, broadening its clinical applicability. In vitro photocytotoxicity assays were conducted to compare the formulated and non-formulated compounds, and in vivo biodistribution studies were performed to evaluate drug distribution. Additionally, the optimal drug-to-light activation interval for Ru-MTI was determined.

Results. The optimized Ru-MTI formulation demonstrated enhanced solubility, preventing aggregation and improving stability for intravenous administration. Biodistribution studies confirmed that Ru-MTI, when administered intravenously, distributed efficiently to targeted organs without inducing noticeable toxicity at the working concentration. The primary screening on the zebrafish model was critical in identifying responsive tumors, which informed further testing of the prodrug in more advanced models. These findings emphasize the importance of formulation advancements and primary screening in enhancing the clinical viability of PACT therapies.

Conclusions. Ru-MTI, with its improved formulation, is a promising treatment for uveal melanoma. The advancements in solubility, aggregation prevention, and stability, combined with the ability for intravenous administration, position this prodrug as a potential candidate for future clinical trials.

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Impact of Cancer-Associated Fibroblasts on the response to Photodynamic Therapy in Oral Squamous Cell Carcinoma spheroid models

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Keywords: cancer-associated fibroblast, photodynamic therapy, cell migration, spheroids

Oral squamous cell carcinoma (OSCC) is one of the most common and aggressive subtypes of head and neck cancer, characterized by its invasive nature and potential for metastasis. Components of tumor microenvironment, particularly cancer-associated fibroblasts (CAFs), play a pivotal role. These fibroblasts interact dynamically with tumor cells, promoting progression and resistance to therapies, including photodynamic therapy (PDT), which can be used in OSCC treatment.

The objective of this study was to develop three-dimensional *in vitro* models (spheroids) that accurately mimic both *in situ* and invasive OSCC to better understand tumor progression and responses to treatment. The models incorporated SCC cell lines from tongue carcinomas (SCC-9, HN-5, and SCC-25) and CAFs isolated from patient biopsies. CAFs were characterized by the expression of key markers (GAL-1, endoglin, α-SMA, vimentin, fibronectin). Confocal microscopy confirmed that the spatial organization of tumor cells and CAFs in the generated models reflected both *in situ* and invasive carcinoma structures. Migration and therapeutic response assays were performed to evaluate the impact of CAF presence on tumor cell behavior and resistance to PDT with Temoporfin.

The results demonstrated that CAFs significantly increased migration in HN-5 and SCC-25 cells, both of which express epithelial-mesenchymal transition (EMT) markers such as vimentin and N-cadherin, compared to SCC-9 cells, which displayed minimal EMT marker expression and high β-catenin localization at the membrane [1]. This migration enhancement was more pronounced in the invasive model, with CAFs isolated from aggressive tongue tumors promoting the greatest migration levels. Additionally, CAFs conferred substantial resistance to PDT, especially in the invasive model, aligning with previous evidence of CAF-mediated therapy resistance [2].

In conclusion, the developed spheroids models successfully replicate the heterogeneity and therapeutic resistance observed in OSCC, underscoring the critical role of CAFs-tumor cell interaction in promoting cell migration and resistance to PDT. Future studies should aim to identify the specific signaling pathways that contribute to these processes, as understanding them could provide insights into novel therapeutic approaches.

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Pre-treatment with topical 5-fluorouracil increases the efficacy of daylight photodynamic therapy for actinic keratoses – a randomized controlled trial

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Keywords: Actinic keratosis, daylight photodynamic therapy, 5-fluorouracil, combination therapy, side effects

Daylight photodynamic therapy (dPDT) and topical 5-fluorouracil (5-FU) are each effective treatments of thin actinic keratosis (AKs). However, efficacy is significantly reduced when treating thicker AKs and prolonged topical treatment regimens can be associated with severe local skin reactions and low compliance. As field-cancerization and AKs are chronic conditions, with recurrences and new AKs commonly observed after treatment, long-term treatment efficacies are crucial.

The aim of this study was to compare the efficacy of sequential 4% 5-FU and dPDT with dPDT monotherapy in the treatment of multiple actinic keratoses in the face and scalp.

Sixty patients with a total of 1547 AKs (grade I: 1278; grade II: 246; grade III: 23) were treated in two symmetrical areas (mean size 75 cm^2) of the face or scalp which were randomized to i) 4% 5-FU creme twice daily for 7 days before a single dPDT procedure and ii) dPDT monotherapy. Daylight exposure was either outdoor natural light or indoor artificial daylight.

Twelve weeks after treatment 5-FU+dPDT resulted in 87% complete lesion clearance compared to 74% for dPDT alone ($p<0.0001$). Especially for moderate-thick AKs, combination treatment improved efficacy (79% vs. 55%, $p=0.0056$). The recurrence rate and number of new AKs within the treatment area were lower for combination treatment than for dPDT alone. Pre-treatment with 5-FU resulted in moderate to severe erythema before dPDT in half of the patients. Moderate/severe erythema were more common in 5-FU+dPDT areas (88%) compared to dPDT (41%) areas two days after dPDT. Twelve months after treatment, 85% of patients were very satisfied with 5-FU+dPDT, compared to 71% after dPDT alone ($p=0.0176$) and stated better efficacy as the reason.

Sequential 5-FU and dPDT was more effective than dPDT monotherapy in the treatment of AKs in the face and scalp, especially for grade II AKs. Better efficacy was maintained 12 months after treatment. Local skin reactions were more pronounced after combination treatment, but no patients discontinued the treatment. The combination of 5-FU and dPDT is an effective treatment of large treatment areas with high compliance and satisfaction.

Image-Guided, Alcohol-Based Photodynamic Therapy for Inoperable Cancers

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Keywords: Intratumoral drug delivery; photodynamic therapy; locally advanced tumors

Each year, more than 1 million people die from pancreatic and liver cancer worldwide [1]. While surgery remain the only curative option, fewer than 30% of patients are eligible for these procedures [2,3]. To improve outcomes, new treatments are urgently needed to convert inoperable patients into surgical candidates. Photodynamic therapy (PDT) is an emerging approach for treating locally advanced pancreatic and liver cancers. However, current photosensitizer drugs, delivered intravenously or orally, often have poor intra-tumoral accumulation, with only a small fraction of the dose reaching the target tissue. To address this limitation, we have developed LASEIT (Light-Activatable, Sustained-Exposure Ethanol Injection Technology), a novel formulation designed to improve both the efficacy and safety of PDT for unresectable tumors [4]. LASEIT increases photosensitizer delivery to tumors by up to 40-fold, enhances light propagation through tumor tissue, and reduces procedure time. In preclinical models of hepatocellular carcinoma and pancreatic ductal adenocarcinoma, LASEIT-PDT has demonstrated significant tumor burden reduction and prolonged survival [5]. In this presentation, we will introduce the next-generation image-guided LASEIT formulation which can be tracked in real time using standard clinical imaging modalities. This advancement is intended to accelerate the clinical translation of LASEIT-PDT for treating locally advanced and unresectable tumors.

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Tetrapyrrolic Photosensitizers in Cancer Radiotherapy: Dual Roles as Radiosensitizers and Radioprotective Agents — Current Insights and Future Perspectives

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Keywords: 5-ALA, porphyrins, oxidative stress, radiotherapy

Tetrapyrrolic compounds represent one of the earliest classes of photosensitizers (PSs) utilized to induce oxidative stress in cancer cells upon light exposure and thus enable targeted cytotoxicity while minimizing off-target effects. However, the clinical utility of PSs is constrained by the limited optical penetration of light through biological tissues. Ionizing radiation (IR), which does not have this drawback, is also used to inactivate cancer cells, but the damage it causes is not selective.

Radiosensitizers are substances that enhance the effectiveness of radiation therapy by making cancer cells more susceptible to radiation damage, either directly or through indirect action leading to cellular inactivation. On the other hand, radioprotectors are compounds that protect normal, healthy tissues from the harmful effects of radiation. One of the main acute effects of IR in living systems is oxidative stress [1]. The increased overall activity of photosensitizers is also attributable to elevated oxidative stress; however, the resultant biological effect depends on a complex interplay of the actual compound's structural attributes, photophysical and photochemical behaviors as well as its biodistribution and accumulation properties. Thus, although the radiosensitizing effect of some tetrapyrrolic compounds has been studied for decades and even clinical trials have been conducted [2], the mechanism responsible for the observed enhancement of response or lack thereof is not fully understood. This is partly due to differences in the experimental protocols used, but also due to the heterogeneity of cancer tumors and differences in their oxygenation levels, which is an important factor determining cellular radiosensitivity and therapeutic outcome.

This review will examine the studies on the possible dual role of porphyrin-type compounds as radiosensitizers and radioprotectors, revealing a versatile approach to optimize cancer radiotherapy. It will focus on the use of 5-ALA, which results in intramitochondrial accumulation of protoporphyrin IX, providing an exceptional opportunity to overcome cancer cell defense mechanisms that play an important role in treatment outcome [3]. Current challenges in combined therapy will be outlined and several possibilities for improving its efficacy will be discussed.

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Efficacy and safety of daylight and artificial white light photodynamic therapy verse minocycline for papulopustular rosacea: A single-center, assessor-blinded, randomized, parallel-group, non-inferiority trial

Background: Papulopustular rosacea (PPR) faces challenges with tetracycline side effects and traditional photodynamic therapy (PDT) pain, prompting exploration of daylight PDT (DL-PDT) and artificial white light PDT (AWL-PDT) as alternatives requiring efficacy and safety validation.

Objective: Evaluating the efficacy and safety of DL-PDT versus AWL-PDT for the treatment of PPR.

Methods: This single-center, assessor-blinded, randomized, parallel-group, non-inferiority trial assigned 69 patients with PPR (1:1:1) to the DL-PDT, AWL-PDT, or minocycline groups. Patients received DL-PDT or AWL-PDT once every two weeks for four sessions, or took oral minocycline 100 mg/day for 6 weeks. The primary outcome measure of this study was the number of papules and pustules. Treatment success was defined as a reduction of at least 50% in the number of papules and pustules from baseline, assessed at four weeks after the final treatment. Secondary outcomes included: investigator's global assessment (IGA), clinical erythema assessment (CEA), and change in the Rosacea-specific Quality of Life Scale (Rosacea-QoL) score.

Results: Among 69 patients, 63 completed 10-week follow-up. At endpoint, median lesion reduction was 23.0, 28.0, and 24.5 in the DL-PDT, AWL-PDT, and minocycline groups, respectively ($p=0.3$), demonstrating photodynamic therapy's non-inferiority to minocycline. No significant differences ($p>0.05$) were observed among groups for IGA success (42.9% vs 45.5% vs 60.0%), CEA success (42.9% vs 50.0% vs 55.0%), or Rosacea-QoL improvement (23.5±8.9 vs 25.3±13.3 vs 26.8±13.8).

Limitations: The single-center design and small sample size may affect the generalizability and evaluation of long-term efficacy.

Conclusion: DL-PDT and AWL-PDT are noninferior to oral minocycline for PPR treatment, providing alternatives for patients avoiding systemic medication risks or traditional PDT pain intolerance.

A Blueprint for Far-UVC Use in Airborne Pathogen Control

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Far-UVC (200–235 nm) is a promising candidate for inactivating airborne and aerosolized pathogens in occupied indoor spaces. Unlike conventional germicidal UV (254 nm), far-UVC is strongly absorbed by proteins in the outermost layers of skin and eye tissues, offering a high margin of safety while retaining virucidal and bactericidal activity. This wavelength range may therefore enable continuous disinfection in occupied spaces—if photobiological safety, air chemistry impacts, and real-world efficacy can be robustly understood and managed.

We present a multidisciplinary synthesis of experimental and modeling results that evaluates far-UVC across five domains: disinfection efficacy, photobiological safety, materials compatibility, indoor air chemistry, and exposure-guideline alignment. Far-UVC inactivates a broad spectrum of bacteria and viruses with limited tissue penetration. However, susceptibility estimates for airborne pathogens vary by orders of magnitude across studies, partly due to differences in aerosol medium and particle structure. Likewise, key questions remain about chronic low-dose exposure effects, air chemistry byproducts (e.g. ozone, formaldehyde), and effects on common materials in the built environment.

We describe how dose-response models and CFD-based dose distribution estimates can be used to bridge bench-scale results with room-scale applications, and how far-UVC compares to other air cleaning technologies in terms of Clean Air Delivery Rate (CADR), energy efficiency, and suitability for sensitive environments.

Our analysis highlights key priorities for the photobiology and public health communities: (1) refining action spectra and exposure limits with population-scale safety in mind, (2) developing standards for efficacy measurement in realistic aerosol conditions, and (3) supporting translational research to responsibly scale far-UVC for pathogen control while earning public trust.

Bispecific aptamer-decorated nanoplatforms targeting tumor and stromal cells in breast cancer derived organoids: implications for precision phototherapies

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Keywords: Phototherapies; Nanomaterials; Aptamers

In this study, we propose novel cancer-targeted nanoplatforms for combined photodynamic (PDT) and photothermal (PTT) therapies of triple-negative breast cancer (TNBC) [1]. Multifunctional hybrid PDT/PTT-active nanoparticles were implemented by incorporating an Iridium-based complex - with photosensitizing and luminescent properties - within the polysiloxane matrix of gold-core/silica-shell nanoparticles [2]. The surface of the nanoplatforms was efficiently decorated with nuclease-resistant 2'Fluoro-pyrimidine RNA aptamers (CL4 and Gint4.T), able to specifically bind to surface proteins expressed on TNBC cells (EGFR and PDGFRβ), without recognizing other breast cancer subtypes and non-tumor cells [2]. The targeting specificity, and the synergistic PDT and PTT effects have been assessed by confocal microscopy and cell viability assays, respectively, on different human cell types including mesenchymal subtype triple-negative breast cancer MDA-MB-231 and BT-549 cell lines (both EGFR and PDGFRβ positive), luminal/HER2-positive breast cancer BT-474 and epidermoid carcinoma A431 cells (only EGFR positive) and adipose-derived mesenchymal stromal/stem cells (only PDGFRβ positive). Cells lacking expression of both receptors were used as negative controls. To take into account the tumor-stroma interplay, fluorescence imaging and cytotoxicity were evaluated in preclinical three-dimensional (3D) stroma-rich breast cancer models.

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Temoporfin-mediated photodynamic therapy induces immunogenic cell death on head and neck cancer

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Keywords: Anticancer photodynamic therapy; immunogenic cell death; temoporfin; anticancer immunity.

Introduction: Despite significant advances in the treatment of head and neck cancers (HNC), tumor recurrence remains a clinical challenge, with rates ranging from 25% to 50%[1]. Photodynamic therapy (PDT) is a clinically approved, minimally invasive therapeutic strategy that exerts its therapeutic effects through the generation of reactive oxygen species[2]. This pro-oxidative mechanism enables many photosensitizers to trigger immunogenic cell death (ICD), a regulated form of cell death that not only eliminates cancer cells but also activates the immune system, promoting long-term anti-tumor immunity. ICD is initiated by a combination of oxidative and endoplasmic reticulum (ER) stress, leading to the release of adjuvants and the exposure of tumor antigens. These signals recruit dendritic cells (DCs), which process tumor antigens and stimulate T cells to establish immunological memory[3]. Among clinically approved photosensitizers, temoporfin (mTHPC) is used for the palliative treatment of HNC. While effective in tumor debulking, its long-term therapeutic efficacy remains inconsistent. This discrepancy may be linked to an incomplete understanding of its ability to induce ICD. Previous *in vitro* studies have shown that mTHPC exhibits time-dependent intracellular localization: initially accumulating in the Golgi apparatus and mitochondria, and later localizing predominantly in the ER after extended incubation[4]. Given the central role of ER stress in ICD, these findings suggest that the interval between drug administration and light irradiation may critically affect treatment outcomes.

Aim: This study investigates whether mTHPC-mediated PDT can induce ICD in murine HNC cell lines, and whether different incubation times influence this response.

Materials and Methods: MOC2 and MOC22 were incubated with mTHPC for 2 or 24h, washed, and irradiated using red LED light (630 nm, 4.12 J/cm^2). All analyses were performed by flow cytometry.

Results: The two cancer cell lines exhibited similar sensitivity to the cytotoxic effects of mTHPC; however, higher concentrations were required to achieve comparable levels of cell death following a 2h incubation compared to 24h. Interestingly, only MOC22 cells showed phosphatidylserine exposure prior to membrane permeabilization, suggesting that this cell line undergoes a form of regulated cell death. However, and although to a lesser extent compared to MOC22, MOC2 cells also showed increased activation of the apoptotic reporter PARP after mTHPC treatment, indicating a possible mixed cell death. We then evaluated the potential of mTHPC to induce ICD by performing a DC maturation assay. We found that both cell lines promoted DC maturation, as evidenced by the upregulation of CD40, CD86, and MHC-II, only when mTHPC was incubated for 24h, supporting our hypothesis. Further *in vivo* studies are underway to confirm mTHPC's ability to induce ICD.

Conclusions: Our findings demonstrate, for the first time, that mTHPC can induce ICD and show that this ability depends on its localization within tumor cells, which is a time-dependent process. These results underscore the importance of optimizing PDT protocols—particularly drug incubation time—to fully harness the immunostimulatory potential of mTHPC in inducing ICD and enhancing long-term therapeutic outcomes.

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Cyto/hemocompatible magnetic hybrid NPs made of iron oxide/silver sulfide quantum dots with luminescence in the near-infrared region for combinational therapy of peritoneal metastasis

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Keywords: Peritoneal metastasis, iron oxide, **s**ilver sulfide, hyperthermia, nanothermometry

Colorectal carcinoma (CRC) is the second most common cancer in Europe and the U.S., with high mortality due to metastasis to the peritoneum, liver, and lungs[1,2]. Peritoneal metastasis (PM) marks a terminal stage, with median survival of 16 months despite chemotherapy. Advanced treatments include cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (HIPEC) and pressurized intraperitoneal aerosol chemotherapy (PIPAC) for unresectable PM. HIPEC faces major limitations: (a) short drug exposure (30–90 min), (b) passive diffusion, and (c) poor tumor selectivity, leading to toxicity, 0–4.1% mortality, and 20–60% morbidity[1,3]. Therefore, improved detection and targeted NP-based therapies are critical.

Light- and magnetic-based nanoparticle (NP) therapies offer tumor specificity, no drug resistance, and minimal invasiveness[4]. NanoHyperthermia (nanoHyp) uses light-activated photosensitizers to generate heat; magnetic nanoHyp uses alternating magnetic fields to induce NP heating without depth limits. Iron oxide (IO) and Ag_2S (AS) NPs are effective near-infrared (NIR) nano-heaters, though high IO doses limit clinical use[4]. This study aims to monitor intracellular temperature using luminescent AS NPs with a nanothermometer (NTm)/nano-heater (NH) hybrid. Cisplatin (Cpt), effective in HIPEC, is limited by renal toxicity and resistance. NP-based delivery improves its efficacy[5]. Cetuximab (Cet), an FDA-approved antibody against EGFR, increases specificity in ~80% of CRC cases. Functionalized NPs (AS-IO-Cet) enhance tumor targeting and reduce toxicity[2]. This work combines (1) tumor targeting, (2) luminescent imaging, (3) precision heating, (4) local temperature sensing, (5) remote activation by magnetic/NIR stimuli, and (6) controlled drug release.

Cet-functionalized AS-IO NPs allow targeted uptake and pH-sensitive Cpt release, combined with nanoHyp, for novel PM therapy. The study uniquely integrates NTm/NH in NIR-II imaging-guided therapy. Hybrid AS-IO NPs were synthesized by conjugating AS NPs (coated with Glutathione, 11-mercaptoundecanoic acid, or PEG) to IO nanocubes via EDC/NHS or ligand exchange. Glutathione-coated AS showed successful IO binding. Cet conjugation followed prior work on AS NPs with Cet and aminolevulinic acid for targeted photodynamic therapy. Cpt was electrostatically loaded at pH 10 (NaOH 1M), and final AS-IO-Cet-Cpt NPs luminesced at 925 nm with a 16.65 nm hydrodynamic size. AS enables nanothermometry, though reliability and reversibility remain concerns. Its temperature-dependent luminescence allows high signal-to-noise readouts, applied to AS-IO hybrids under magnetic hyperthermia. EGFR targeting by Cet-NPs will be tested in EGFR(+) CT26 and EGFR(-) HT29 cells (4 and 24 h) using confocal microscopy and ICP-MS. Short incubation will help distinguish receptor-mediated endocytosis from passive uptake—addressing selectivity and toxicity issues in cancer therapy.

Initial data showed minimal AS-IO and AS-IO-Cet toxicity up to 100 µg Ag/mL. Magnetic hyperthermia effects and luminescent temperature measurements will provide precise intracellular NP temperature data. Structural changes, localization, NP quantification (flow cytometry, high-content screening, EPR), and TEM will assess lysosomal confinement, aggregation, degradation, and recrystallization. Lysosomal stress will be evaluated via immunohistochemistry and high-content microscopy.

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Activatable Photosensitizers: From Molecules to Nanoparticles

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Keywords: Activatable Phototherapy, Porphyrin, Nanoparticles, Cancer imaging and treatment

Photodynamic therapy (PDT) is a treatment modality that utilizes a light-activated photosensitizer, in combination with molecular oxygen, to generate cytotoxic reactive oxygen species for localized tumor destruction. In 2004, our laboratory introduced the concept of activatable photosensitizers, in which singlet oxygen generation remains quenched until disease-specific molecular interactions activate the photosensitizer, thereby significantly enhancing treatment selectivity and minimizing off-target effects[1]. In pursuit of nanoparticle-based activatable systems, we discovered porphysomes in 2011—liposome-like nanostructures self-assembled from porphyrin–lipid conjugates. The densely packed porphyrins in the porphysome bilayer enable efficient light absorption and thermal conversion, making them highly effective for photothermal therapy and photoacoustic imaging. Upon cellular uptake and disassembly, the porphyrin monomers regain their fluorescence and PDT activity[2]. Moreover, porphysomes can be chelated with metal ions, extending their utility to PET and MRI imaging[3]. After more than a decade of development, porphysomes are now poised for first-in-human clinical translation. We have subsequently developed next-generation porphysomes with enhanced biocompatibility and expanded theranostic functionality, including applications in radionuclide therapy and photochemically induced immune stimulation. In a syngeneic CT-26 colorectal tumor mouse model, we demonstrated that porphysome-mediated PDT not only achieves effective tumor ablation but also elicits systemic anti-tumor immune responses. These advancements establish a new paradigm for porphysome applications—extending beyond light and beyond local treatment.

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Supramolecular photosensitizers based on oligo- and polysaccharides: from self-assembly to applications in prevention and control of antibacterial infections

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Keywords: Porphyrinoids, Curcuminoids, Cyclodextrins, Supramolecular Photosensitisers, Hyaluronic Acid.

New prevention and control strategies combined with innovative resorbable antimicrobial biomaterials development are urgent to fight emerging infection in the treatment of joint arthroplasty complications or surgical wounds by overcoming the multidrug resistance (MDR). One of the approaches points to the design of smart supramolecular photosensitisers with antimicrobial photodynamic therapy (aPDT) action. Due to depleted photostability of free photosensitiser (PS), the development of novel supramolecular constructs are currently necessary to avoid PS photodegradation, thus improving the photo-antibacterial efficacy. Within our ongoing research on nano- and micro- photosensitisers [1], here we will discuss our recent advances on hydrogels incorporating curcuminoids, to prevent and control joint infection, or porphyrinoids for treatment of traumatic wounds. In particular Curcumin I (Cur) and Vancomycin (Van) co-loaded hydrogels were prepared and fully characterized [2]. The drug association demonstrated the best activity against MRSA with respect to the same hydrogel loaded with high amounts of Van, used during the surgery, due to the photo-antibacterial activity of Cur combined with the antibiotic action of the low dose of Van. On the other hand, hydrogels containing nanocomplexes made of the zinc(II) derivative of 5,10,15,20-Tetrakis(1-methylpyridinium-4-yl)porphine tetrakis(p-toluenesulfonate) (ZnTMPyP) and the trade sulfobutylether-β-cyclodextrin (CAPTISOL®) were formulated for the treatment of wound infections. These systems exhibited photo-bactericidal activity against Gram-positive and Gram-negative bacteria by revealing their aPDT properties. Altogether the proposed photosensitizing hydrogels are experienced as promising candidates for the treatment of the periprostetic joint and surgical wounds infections.

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Acknowledgements:

This work was supported by the MUR-PNRR through the projects SAMOTHRACE (SiciliAn MicrOnanoTecH Research And innovation Center) ECS 00000022.

Triplet Lifetime Matters: Designing Photosensitizers for Effective Photodynamic Therapy in Hypoxic Tumors

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Keywords: photodynamic therapy; triplet state; hypoxia; bacteriochlorins

Photodynamic therapy (PDT) is a promising approach for treating solid tumors, yet its effectiveness is often limited by hypoxia within the tumor microenvironment (TME). The efficiency of PDT under hypoxic conditions critically depends on the generation of reactive oxygen species (ROS), which is governed by the triplet state lifetime of the photosensitizer (PS).[1] In this context, triplet state engineering emerges as a vital strategy. Traditionally, incorporation of heavy metal ions (e.g., Zn(II), Pd(II), Pt(II)) into porphyrin macrocycles is employed to enhance intersystem crossing (ISC) and triplet state quantum yields. However, increasing atomic number generally leads to shorter triplet lifetimes, potentially reducing singlet oxygen quantum yield and overall PDT efficacy in low oxygen environments. Our results emphasize that metal-free porphyrins functionalized with multiple halogen atoms (F, Cl,) may offer a superior alternative. These halogenated PSs display prolonged triplet lifetimes, enhanced ROS generation capacity, and the potential to engage in alternative photochemical mechanisms such as photoinduced electron transfer. In our studies, bacteriochlorins (F_2BOH, F_2BMet, Cl_2BHep, F_2BPrc) with varying lipophilicity were evaluated in BALB/c mice bearing CT26 tumors under vascular-, endothelial-, and cellular-targeted PDT protocols (V-PDT, E-PDT, C-PDT). F_2BOH and F_2BMet showed >80% cure rate in V-PDT, while Cl_2BHep achieved 100% survival in C-PDT with a low light dose of 45 J/cm². Inflammatory profiling revealed IL-6 as a key mediator of PDT-induced immune response.[2]

Furthermore, hypoxia dynamics were evaluated in a Lewis lung carcinoma (LLC) model using F_2BMet (redaporfin). Short DLI (15 min) V-PDT protocol significantly decreased tumor oxygenation and blood flow, leading to durable antitumor effects in 67% of animals. Real-time pO_2 and perfusion monitoring demonstrated that appropriate vascular targeting can maximize therapeutic outcomes, even under hypoxia.

Collectively, these findings highlight that rational design of PSs with long-lived triplet states can sustain Type I and II photochemistry under hypoxic conditions. Halogenated porphyrinoids emerge as safe and effective candidates, challenging the conventio that heavy metals are essential for PS optimization in PDT. This paradigm shift supports the development of clinically viable, metal-free PSs tailored for use in hypoxic tumors.

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How near is the red? Phthalocyanines as triplet photosensitisers

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Keywords: Phthalocyanine; Photodynamic; Photosensitiser; Phototherapeutic window

Phthalocyanines have strong maximum absorption that can be adjusted between 660 and 800 nm (and even up to longer wavelengths for peculiar derivatives). This matches perfectly the phototherapeutic window. Many challenges remain however to produce perfect phthalocyanine-based photosensitisers: biocompatibility and tumour targeting being the most crucial nowadays.

Our latest works aiming at improving the overall efficiency of phthalocyanine-based nano-photosensitisers will be presented, with either self-encapsulation in phthalocyanine-centered polycaprolactone star-polymers [1], formulation with various micellar agents [2-3], or inclusion into biopolymers [4-5].

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Platinum(II) porphyrin metal-organic frameworks: luminescent tools for optical oxygen sensing in biomineralisation-capable bacteria biofilms

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Keywords: Platinum(II) porphyrins; Oxygen sensing; Biomineralization

The ability to repair cracks on concrete surfaces plays a vital role in extending the longevity of concrete structures, reducing the maintenance costs and carbon footprint of built environment.

Recently, biomineralization-based techniques attracted interests as a Nature-inspired solution to generate self-healing building materials. Biomineralization relies on the ability of bacterial to deposit calcium carbonate and seal micro-cracks on concrete surfaces. This phenomenon, called microbially-induced calcite precipitation (MICP), has been shown to prevent the expansion of the crack, thereby delaying the degradation that leads to structure failure [1,2]. Progress of biomineralization-based technology depends on the availability of analytical tools to monitor the viability of MICP-capable bacteria expeditiously and in real time.

In this work, we report new optical oxygen sensing agents based on metalloporphyrin-containing metal organic frameworks (MOFs) to monitor the levels of oxygen in *Shewanella oneidensis* biofilms.
Four oxygen sensing porphyrins were obtained from the platinum complex of tetrakis-5,10,15,20-pentafluorphenylporphyrin by thiol-fluoride or oxygen-fluoride aromatic nucleophilic displacement [3]. The resulting species were incorporated into MOFs structures, as the sole ligand or in conjunction to 1,4-benzenedicarboxylic acid to generate doped species. We will discuss the oxygen sensing potential of these species in solution and as tools to monitor oxygen consumption in MICP-capable bacteria colonies.

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Bioluminescence-based Singlet Oxygen Generation

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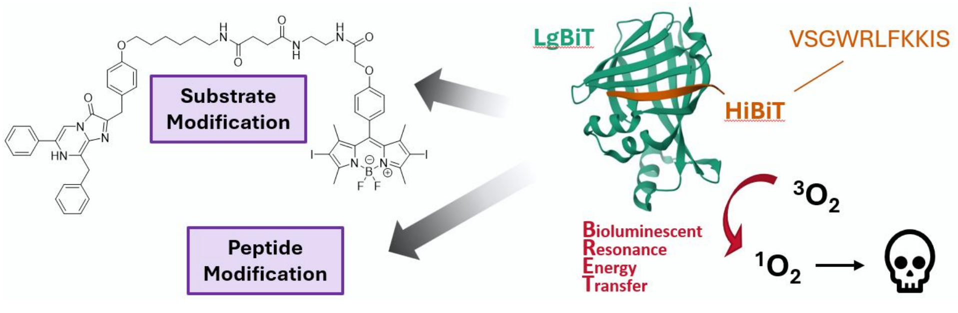
Keywords: Singlet Oxygen; Photosensitizer; Bioluminescence Resonance Energy Transfer (BRET); NanoBiT; Coelenterazine

Bioluminescence resonance energy transfer (BRET) can be used to excite suitable acceptors such as photosensitizers leading to the generation of singlet oxygen ($^1O_2$), which is cytotoxic with a short lifetime of ~3-0.01 µs [1] and, therefore, a limited diffusion range (maximum of 134 nm *in vivo* [2]). This self-illuminating approach is independent of external excitation, which reduces heat conversion and photobleaching, enabling longer homogeneous measurements. BRET-based singlet oxygen generation can either be utilized for the specific inactivation of biomolecules, the mapping of biopolymer interactions or even lead to cell death, which e.g. extends the scope of photodynamic therapy (PDT).[3] Based on a split version of the semisynthetic luciferase NanoBiT, i.e., the LgBiT protein and the 11-mer HiBiT peptide, we report two self-illuminating systems that aim at producing $^1O_2$. On the one hand, the NanoBiT substrate coelenterazine was conjugated to halogenated BODIPY moieties with different linker lengths. Michaelis-Menten constant determination demonstrated that our long linker compound could be oxidized by the NanoBiT and, in turn, emit light. Although bioluminescence emission was lower than the one from the unmodified substrate, the BRET was successfully measured.[4] BRET-based generation of $^1O_2$ could only be detected *in vitro* using Si-DMA[5], but not *in cellulo*. On the other hand, we are currently working on a different approach based on HiBiT modification that overcomes the previous limitations in cellular setups.



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Modification of the efficiency of melanin to photogenerate singlet oxygen by selected quenchers of triplet excited states.

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Keywords: melanin; photoreactivity; singlet oxygen; excited triplet states

Photoreactivity of retinal pigment epithelium melanin increases with age of the donors (Olchawa et al., 2021), and we have demonstrated that in vitro photoaging of synthetic melanins dramatically enhances their efficiency to photogenerate singlet oxygen (Zadlo et al., 2019; Mokrzynski et al., 2024). In this work, we analyzed the effects of reversible and degradative oxidation of synthetic pheomelanin and eumelanin on selected parameters of their excited triplet states responsible for photogeneration of singlet oxygen. Water soluble melanins obtained by autooxidation of dopa and enzymatic oxidation of 5-S-cysteinyldopa were used as synthetic models of eumelanin and pheomelanin, respectively. Reversible oxidation of the melanins were obtained by their titration of potassium ferricyanide while oxidative degradation was achieved by aerobic photolysis of the melanin with intense violet light. Triplet excited states of the melanins were monitored indirectly by measuring the degree of quenching of singlet oxygen photogenerated by control and photobleached melanins, induced by potassium sorbate, sorbic alcohol and benzoic acid used as selective triplet state probes of melanin. The obtained data indicate that photoexcitation of melanin with near UV and short wavelength visible light leads to the formation of a population of excited triplet states, which are only partially quenched by the employed probes, suggesting that energies of the quenchable triplet excited states are higher than 200 kJ/mol, while the remaining triplet states that could not be quenched by the probes, have lower energies. Based on a simple kinetic model, sorbic alcohol quenched the excited triples states of melanin with bimolecular rate constant (0.5-1.7)x10^8 M^{-1} s^{-1}. Reversible oxidation of eumelanin and pheomelanin resulted in a reduction of the melanin ability to photogenerate singlet oxygen. Therefore the enhanced efficiency of photodegraded melanin to photogenerate this reactive oxygen species must be due to specific oxidative modifications of the melanin monomers and fragmentation of the melanin aggregates. Stronger reduction of the efficiency of photodegraded melanins to photogenerate singlet oxygen, induced by the employed triplet probes, suggests an increased accessibility of the photodegraded melanin triplet sites to the probe molecules and formation of excited triples states with higher energies. It can be concluded that efficient protection against elevated photoreactivity of aged melanins may involve application of appropriate quenchers of the melanin triplet excited states. Supported by National Science Centre (project OPUS-22 2021/43/B/ST4/03255).

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The Purple Power in Europe: Purple Phototrophic Bacteria for Resource Recovery from Organic Waste Sources

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Keywords: Photo-biorefineries, purple photosynthetic bacteria, circular bioeconomy

The transition toward a circular bioeconomy demands the development of novel, sustainable biorefineries capable of recovering valuable resources from organic waste streams. Within this context, **Purple Phototrophic Bacteria** (PPB) have emerged as versatile biocatalysts, capable of transforming diverse organic substrates into high-value products under light-driven conditions.

At **Universidad Rey Juan Carlos** (URJC), we are actively advancing the concept of **photo-biorefinery** through applied research that demonstrates the technical and environmental potential of PPB-based processes. Our recent developments include:

- A photo-electroautotrophic system for integrated nitrogen recovery from pig slurry, biogas upgrading, and microbial protein production.
- The bioconversion of oily sludge from petrochemical industries into edible bacterial biomass.
- The upcycling of PET plastic waste into bioplastics (polyhydroxyalkanoates).
- Novel approaches to bioelectrochemical valorization of wastewater, leveraging the electroactivity of PPB.

These systems reflect our ongoing efforts to deliver scalable and decentralized solutions for waste valorization. A milestone in this direction is the development of demonstration-scale facilities, particularly the one implemented in Linares (Jaén) under the **DEEP PURPLE** project, which is now being replicated and expanded in Badajoz. These facilities integrate PPB-based photobioreactors into municipal waste and wastewater treatment infrastructure, offering a blueprint for near-market applications.

While Europe has a rich legacy of innovation in this field — including foundational projects such as MELiSSA and other initiatives that have successfully concluded— new projects are paving the way forward. Notably, current EU-funded efforts focus on:

- The production of PPB biomass as a functional ingredient for food and feed applications.
- The development of decentralized water-sensitive technologies and new governance models using PPB-based systems.

These initiatives are aligned with broader efforts to industrialize PPB technologies and unlock their full potential in sun-rich regions.

Finally, the **COST Action PurpleGain** plays a key role in connecting stakeholders across 36 countries, promoting scientific exchange and technological harmonization throughout the PPB value chain, and supporting the advancement of photo-biorefinery concepts from research to real-world deployment.

Upcycling agri-food by-product with phototrophic purple bacteria

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Keywords: PPB, Health-promoting ingredient, Agri-food byproducts

Introduction: Phototrophic Purple Bacteria (PPB) are very promising organisms for the development of resource recovery strategies and circular biobased economy. Indeed, their very versatile metabolism make them capable of using a very large variety of substrates such as carbon, nitrogen and electron sources. When growing phototrophically, these organisms reach the maximum nutrient conversion yield of one, allowing for very efficient waste stream treatment and resource recovery.

Context: In our laboratory, we are studying the production of PPB as innovative health-promoting ingredient for feed and food applications. In this case, waste stream have to be properly selected in order to ensure food-grade quality. Agri-food industry produces very large amount of food grade waste or by-product which are today very poorly valorized [1]. Indeed, most of the today existing valorization strategy consist in energy or fertilizer production which represent a decrease of the intrinsic value of the product. We prefer to favor “up-cycling” valorization scheme in which the value of the product is increased. It is notably the case with the use of sugar-beet factory molasses for the production of PPB. Hence, molasses contains essentially sucrose but can be used to produce biomass of PPB which is not only protein rich but also contains anitoxydant and vitamins.

Results: We have recently optimized the use of *Rhodospirillum rubrum* for the valorization of sucrose containing agri-food waste. The strain has been adapted to the assimilation of glucose which is impossible in the WT strains. A cultivation process has been developed which use low cost photobioreactor in order to decrease significantly capital expenses. Today the cultivation process is being optimize to tune the metabolism of PPB to maximum carbon conversion yield and the production of health-promoting component.

Conclusion: The use of low cost production facilities for production of biomass of PPB to be used as innovative and health-promoting feed and food ingredients today appears as a sustainable and economically viable resource recovery strategy for agri-food waste vaorization.

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Harnessing purple non-sulfur bacteria for gas to protein bioconversion

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Keywords: Hydrogen; Nitrogen; Resource recovery; Single cell protein

Microbial protein (MP), particularly from renewable feedstocks, provides a sustainable solution to conventional energy-intensive food production systems. Purple non-sulfur bacteria (PNSB) emerge as a promising microbial platform due to their exceptional metabolic versatility. They can grow phototrophically on waste-derived organic compounds and gaseous substrates such as H_2 and CO_2, enabling their integration into gas-based biorefineries. PNSB also possess the ability to fix N_2 when soluble N sources in the medium are scarce. Their high biomass yields, up to 1 g $COD_{biomass}\cdot$g $COD_{consumed}^{-1}$, and the favorable essential amino acid profile of PNSB biomass make them a valuable source of MP, suitable for animal feed with potential for human nutrition [1]. Unfortunately, photoautotrophic and diazotrophic metabolisms of PNSB remain largely underexplored regarding their application in resource recovery. The capability of PNSB consortia to grow on H_2 and CO_2, as well as under diazotrophic conditions was explored.

MP production by an enriched PNSB consortium from H_2 and CO_2 was investigated, assessing the influence of environmental parameters (pH 6-8.5, temperature 15-50°C and light 0-50 $W\cdot m^{-2}$). Optimal bioconversion conditions of pH 7, 25 °C and light intensities >30 $W\cdot m^{-2}$, were identified. At these favorable parameters, high biomass yields (~1 g $COD_{biomass}\cdot$g $COD_{consumed}^{-1}$) and protein yields (~4 g protein$\cdot$g H_2^{-1}) were achieved. The resulting biomass consistently exhibited high protein content (51-64% $w\cdot w^{-1}$) and adequate amino acid profile, confirming its suitability as feed [2]. Additionally, N_2 fixation by enriched PNSB cultures was evaluated under heterotrophic conditions using volatile fatty acids and under autotrophic conditions with CO_2 and H_2. Enrichments yielded cultures with efficient N_2 uptake, achieving near-complete N recovery into biomass. COD biomass yields averaged 0.88 g $COD_{biomass}\cdot$g $COD_{consumed}^{-1}$, lower compared to those obtained using ammonium. Notably, N_2 fixation with acetate showed high substrate uptake rates and produced biomass with over 44% crude protein [3].

These studies highlight the potential of enriched PNSB consortia for sustainable protein production. Their ability to grow on diverse feedstock, supports alternative food and feed systems, reducing dependence on intensive agriculture and synthetic fertilizers.

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Microbe-electrode interfaces for sustainable technologies

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Keywords: Microbial electronics; Cyanobacteria; Biophotovoltaics;

Microbial metabolisms offer large reaction networks that can be exploited for both energy and chemical production in bio-electrochemical devices. These systems rely on extracellular electron exchange between microbes and electrodes. Catabolic processes can fuel electron transfer from microbes to electrodes for bioelectricity generation, while anabolic processes can benefit from an electron influx for intracellular regeneration of reducing equivalents. This versatility enables a wide range of applications, including bioremediation, biosensing, and chemical production. However, electron transfer between electrodes and microbes is often limiting, thus negatively impacting device performances [1].

We address this bottleneck from both biological and electrode engineering perspectives. Our work focuses on engineering microbes and electrodes using nanoparticles [2,3], biosynthetic and conductive polymers [4-6], as well as the heterologous expression of extracellular electron transfer pathways [7-9]. These advances in improving the electrode-microbe interactions led to increased electricity generation in microbial fuel cells for wastewater treatment and in bio-photovoltaics for solar energy conversion. The combination of these cross-disciplinary advances thus pushes forward the development of efficient microbial bioelectronic technologies.

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Photosynthetic bacteria-based electrolysis for green hydrogen production

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Keywords: Anoxygenic Photosynthetic Bacteria; Photo-biocathode; Microbial Electrolysis; Green Hydrogen;

Photosynthetic purple non-sulfur bacteria (PNSB) exhibit a versatile metabolism, making them valuable biocatalyst in biohybrid electrochemical systems for diverse applications like biosensing, wastewater remediation, energy generation, and chemical production.[1] Of particular interest is their use as bioelectrocatalysts for green hydrogen production with concomitant wastewater treatment, contributing to the decarbonization of the energy sector.[2] Herein, we report the development and characterization of a photo-biocathode based on polyhydroxybutyrate-carbon nanofibers, coated with a biofilm of polydopamine and purple bacterial cells, *Rhodobacter capsulatus*, for the photo-driven production of H_2.[3] Characterization techniques, including scanning electron microscopy, cyclic voltammetry, and chronoamperometry, revealed the significant contribution of PNSB to the observed cathodic photocurrent. Further, the possibility of modulating H_2 production by means of applied electrode potential is also discussed. The light-enhanced catalytic activity of the biohybrid photocathode is herein discussed together with the future research directions for paving the way for its applications in water remediation and hydrogen production.

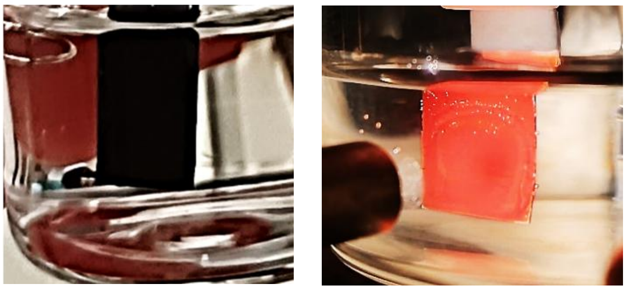

Figure 1. Left) abiotic polyhydroxybutyrate-carbon nanofibers cathode. Right) PNSB-modified photocathode during hydrogen evolution.

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Chemical Engineering of Living Cell Surfaces via Melanin-Inspired Coatings for Biotechnological and Environmental Applications

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Keywords: diatoms, polydopamine, photosynthesis, bioremediation

Photosynthetic microalgae, especially diatoms, offer a promising and self-sustaining solution to climate and pollution challenges due to their natural ability to fix atmospheric CO_2 while producing valuable biomass.

Our work focuses on the bioinspired chemical engineering of living cell surfaces to develop versatile and responsive biohybrid systems.[1] We use melanin-like polymers—specifically polydopamine (PDA), a poly-hydroxyindole compound—to achieve spontaneous and versatile surface functionalization in mild, aqueous conditions. This coating preserves cell viability and proliferation while increasing resistance to environmental stress.[2]

The PDA-coated cells present highly reactive surfaces that can undergo further functionalization through selective reactions with various groups. We have shown that copolymerization of dopamine with boronic acid-containing compounds leads to coatings capable of recognizing saccharide structures, opening possibilities for applications in cell–cell recognition and biosensing.[3]

Our approach is particularly focused on diatom-based systems for environmental remediation. As a case study, diatom biofilms grown on boronic acid-functionalized glass substrates exhibit improved adhesion and can remove heavy metal ions from contaminated water, demonstrating potential for reusable bioremediation platforms.[4]

In this context, we also aim to modify the surface chemistry of naturally CO_2-fixing diatoms, to develop advanced systems that combine carbon capture with selective pollutant removal. This work highlights how the integration of bioinspired materials with phototrophic organisms can contribute to innovative biotechnological solutions for environmental challenges.

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DNA damage remodels the MITF interactome to increase melanoma genomic instability

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Keywords: MITF; DNA Damage repair; Melanoma; Homologous Recombination

Multicellular organisms have evolved remarkable tissue-specific functional specializations that promote the survival, sometimes at the expense of specific tissues. While DNA damage repair is considered a fundamental cellular function across all cell types, it is not known whether cells which are normally exposed to high levels of DNA-damaging agents (such as those in the skin) have evolved lineage-specific mechanisms to enhance organismal survival.

The Melanocyte Inducing Transcription Factor (MITF) is essential for melanocyte development and regulated expression of melanin synthesis, which protect the skin from UV-induced DNA damage. Reinforcing its role in UV protection, MITF also transcriptionally regulates DNA damage repair genes, helping to explain why dedifferentiated MITF^{Low} melanoma exhibit increased tumour mutation burden (TMB).

Unexpectedly, analysis of melanoma samples from the International Cancer Genome Consortium revealed that tumors in the highest quintile of MITF expression harbored the highest TMB. Using laser micro-irradiation, we demonstrated that DNA double-strand break-inducing agents trigger ATM-dependent phosphorylation of MITF at S325. This phosphorylation competitively displaces MRE11 from the MRE11–RAD50–NBS1 (MRN) complex at DNA damage sites, impairing homologous recombination repair (HRR) in a transcription-independent manner. This mechanism explains the paradoxical increase in genomic instability in tumors with very high MITF expression. Notably, the SUMOylation-defective MITF-E318K mutation mimics the effect of S325 phosphorylation, providing a mechanistic basis for its role in melanoma predisposition.

Away from DNA damaged sites, UV irradiation leads to MITF degradation, resulting in transient melanocyte dedifferentiation and proliferation, as has been seen both in human skin and in zebrafish models. The potential penalty imposed by delayed HRR caused by reduced MRN complex function would be offset by increased melanocyte proliferation and subsequent differentiation. This process is driven by activation of MC1R on melanocytes by UV-activated keratinocytes, leading to elevated MITF expression and activity that protects against future DNA damage.

In summary, our findings provide a key insight into how a tissue-restricted transcription factor and lineage survival oncogene, MITF, can shape the response to DNA damage. These results also provide a mechanistic explanation for the role of the E318K mutation in melanoma predisposition.

Targeting GRPR for sex hormone-dependent cancer after loss of E-cadherin

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The presence of sex inequalities in cancer has been documented, yet our understanding of the fundamental processes involved remains restricted, impeding the development of precision medicine and the exploration of novel therapies. Here we show that women, between puberty and menopause, are more prone than men to develop cancer, including melanoma. Importantly, we identified the cell-cell adhesion molecule, E-cadherin, as a central node of the estrogen response in multiple cancers. Using an original mouse melanoma model, we identify a previously unknown estrogen-sensitizing signaling pathway linking E-cadherin/b-catenin to Estrogen Receptor (ESRa) and Gastrin Releasing Peptide Receptor (GRPR) that promotes melanoma aggressiveness in females. Inhibition of this axis by targeting ESRa or GRPR suppresses metastasis in mice and represents promising therapeutic approaches. Our results demonstrate that E-cadherin prevents the sensitivity of melanoma, and carcinoma, to estrogens, and identify a new therapeutic avenue to treat women with multiple E-cadherin-dependent cancers.

Molecular Mechanisms of Hair Graying: Role of Dicer

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Hair graying results, among other factors, from the depletion of melanocyte stem cells (McSCs) in hair follicles—a process accelerated by stress, whether genetic or environmental. Although the precise link between stress and the loss of McSCs remains poorly understood, molecular mechanisms are beginning to emerge to explain this phenomenon. In this presentation, we will show that β-catenin is regulated by UV light and that one of its direct targets, Dicer, plays a key role in the graying process of hair and skin appendages.

Dicer is an essential enzyme in the maturation of microRNAs (miRNAs), which are crucial regulators of gene expression, acting on the stability and translation of messenger RNAs. We have shown that the inactivation of Dicer, both in vitro and in vivo, disrupts the migration and localization of melanocytes within the hair follicle. This disruption first prevents the transfer of melanin to the developing hair shaft and subsequently leads to a gradual depletion of the McSC reservoir. Among the molecular mechanisms involved, the miR-92b/ItgaV axis plays a decisive role.

In conclusion, these findings open promising avenues for better understanding hair aging and for developing strategies aimed at preserving or restoring hair pigmentation by targeting the identified molecular pathways.

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

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Keywords: melanins, UVA1, pigment darkening

UVA exposure elicits immediate and persistent pigment darkening on the skin that are thought to result from oxidation, polymerization of existing melanin and/or precursors. Nevertheless, these structural modifications have never been characterized in human skin. The aim of this study was to characterize the changes in melanin composition and skin color following UVA1 irradiation, exploring the mechanisms underlying immediate and persistent pigment darkening.

Ex vivo skin samples were exposed to increasing UVA1 (340-400 nm) doses. Colorimetric changes were assessed before, immediately after, and two hours post-exposure using a spectrophotometer to measure L* (luminance), b* (yellow-blue component), and Individual Typology Angle (ITA°). Melanin content and composition were analyzed by spectrophotometry and high-performance liquid chromatography (HPLC) following epidermal-dermal separation. Specific degradation products of eumelanin (PTCA, PDCA, Free PTCA, PTeCA) and pheomelanin (TTCA, 4-AHP) were quantified to assess photo-oxidation, cross-linking, and degradation [1-4].

UVA1 exposure resulted in visible skin darkening, evidenced by a decrease in L*, b*, and ITA° immediately and two hours post-irradiation. HPLC analysis revealed a dose-dependent decrease in the PTCA/A500 ratio and an increase in Free/Total PTCA and PTeCA/PTCA, suggesting both photo-degradation and cross-linking of eumelanin. An increase in the TTCA/4-AHP ratio indicated photo-oxidation of pheomelanin.

These findings provide the first direct evidence of UVA1-induced melanin modifications associated with pigment darkening occurring in human skin. The observed changes in melanin markers suggest that both degradation and oxidative processes contribute to the immediate and persistent pigmentation response following UVA1 exposure.

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The double-edged sword dilemma – photoreactivity and antioxidant action of melanin pigments demonstrated in synthetic and natural melanins

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Keywords: melanin; photoreactivity; phototoxicity; antioxidant

Although melanin is commonly viewed as a natural sunscreen that protects pigmented cells against the adverse effects of solar radiation, recent studies have demonstrated that, under certain conditions, the pigment can actually contribute to light-induced oxidative damage of the cells by exhibiting elevated photoreactivity. Photoreactivity of melanoma has become a major focus of research due to the postulated involvement of the pigment in UVA-induced melanomagenesis. In this research, we have examined both aerobic photoeactivity and the free radical scavenging efficiency of synthetic models of eu- and pheomelanin and human melanins from different sources, including induced pluripotent stem cell-derived melanocytes. The effect of the experimental oxidative photodegradation of selected melanins was also studied. Samples were characterized using EPR, UV-Vis spectrometry, DLS spectrometry and HPLC determination of the melanin degradation products. To examine the photophysical and phoyochemical reactivity of the melanins, EPR oxymetry, EPR spin-trapping and time-resolved singlet oxygen phosphorescence were employed. Antioxidant potential of the pigments was determined by EPR-based DPPH assay and quenching of singlet oxygen photogenerated by Rose Bengal. The obtained data indicate the conditions that could contribute to enhanced photoreactivity of melanin shedding light on the phenomenon of photoxicity of this photprotective pigment.The research was supported by grants from the National Science Centre of Poland (NCN): Sonata-2015/19/D/ST4/01964, Preludium-2020/37/N/NZ1/01054, Opus-2017/27/B/ST5/02631 and Opus-2021/43/B/ST4/03255.

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Amphiphilic fatty acid conjugates of pyridylporphyrins in photodynamic therapy of melanoma cell lines

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Keywords: amphiphilic porphyrins, melanoma, photodynamic therapy, hydrophilic-lipophilic balance

Amphiphilic porphyrins, whose hydrophobic part increases cellular uptake and hydrophilic part enables water solubility, have aroused great interest among scientists for use as photosensitisers (PS) in photodynamic therapy (PDT)[1,2]. However, hydrophilic-lipophilic balance of amphiphilic porphyrins, required to avoid all obstacles of highly hydrophobic or hydrophilic PSs, is somewhat less discussed.

Therefore, the aim of this work is to investigate the hydrophilic-lipophilic balance of four groups of asymmetric AB$_3$ pyridylporphyrins. Porphyrins were prepared by conjugation with alkyl chains of different lengths and by quaternization of the nitrogen on the pyridyl ring. The spectroscopic properties of newly synthesised porphyrins in the ground and excited states, the production of singlet oxygen ($^1O_2$) by photodegradation of DPBF and ABMDMA and their lipophilicity were investigated. *In vitro* studies included the determination of cellular uptake, localisation and (photo)cytotoxicity towards normal and tumour cell lines.

The work is divided into three parts. In the first part, two series of porphyrins, *N*-methylated (pyridinium-3-yl) porphyrins and Zn(II) analogues with alkyl chains of different lengths (7-17 C atoms) were compared. Pyridiniumporphyrins with a 13 C-atom long alkyl chain, especially the Zn(II)-chelated analogue, showed the highest cytotoxicity and selectivity towards the melanoma cell lines (MeWo and A375) when irradiated with orange light ($\lambda = 607$ nm)[2]. The second part comprises the comparison of amphiphilic *N*-oxidised pyrid-3-ylporphyrins with 9, 13 and 17 C atoms of alkyl chain. (Oxido)pyridylporphyrins with an alkyl chain with 13 and 17 C atoms proved to be promising PSs, diminishing the melanin obstacle and showing higher selectivity for pigmented melanoma. In the last part, amphiphilic (pyridinium-3-yl) porphyrins with alkyl chains of 9, 13 and 17 carbon atoms were chelated with ^{68}Ga(III) and ^{nat}Ga(III) to obtain the PET/PDT agents, and their distribution was analysed in PET-CT scan. It was found that the hydrophilic-lipophilic balance is important both at the *in vitro* level, affecting selectivity and cytotoxicity towards tumour cell line, and at the *in vivo* level, where the impact of the hydrophilic-lipophilic balance affects the biodistribution of the PS.

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Tuesday, August 26th

Antimicrobial Photodynamic Therapy for Clinical Applications
Chair: Luis Arnaut

Positioning Antimicrobial PDT Between Antiseptics and Antibiotics

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Keywords: Photodynamic therapy, antimicrobial resistance, diabetic foot infections

Antiseptics were introduced in clinical practice soon after Pasteur developed the germ theory and its application to wound putrefaction. Lister hypothesized that the presence of microorganisms in air was the cause of wound infection and introduced phenol as an antiseptic. However, phenol, and the currently used antiseptics such as chlorhexidine, povidone-iodine (Betadine®) or hydrogen peroxide, show toxicity to human tissues at the concentrations required to eradicate bacteria. Antibiotics revolutionized the treatment of bacterial infections because they offered selectivity indexes (SI) larger than 100. However, the selectivity of antibiotics became their Achilles' heel: as antibiotics become more and more specific, they become increasingly dependent on a specific target, and bacteria more easily adapt to avoid the expression of that target. Bacteria adaptation to antibiotics exposure is at the origin of the current pandemic of antimicrobial resistance.

PDT employs a photosensitizer that absorbs light and interacts with oxygen to generate reactive oxygen species (ROS). The low penetration of light in tissues is often cited as a limitation for PDT, but bacteria in skin infections is not normally seen at a depth more than 1.5 mm from the surface of the wound, and the optical penetration depth of skin for wavelengths above 600 nm is higher than 1.5 mm. A more serious limitation of aPDT is selectivity towards bacteria. The ROS generated by PDT also trigger mammalian cell death, and it is challenging to develop a treatment regime where aPDT inactivates >99.9% of prokaryotic cells in biofilms while sparing >70% of eukaryotic cells [1].

We report our efforts to develop photosensitizers and protocols to increase the selectivity of aPDT to microorganisms [2]. For the same light dose (5 J/cm^2) and [KI]=50 mM, the concentration of photosensitizer that reduces by 99.99% the number of planktonic bacteria colony-forming units (minimum bactericidal concentration [3]) is 50 nM, whereas the EC50 of HaCaT is >1 M, i.e., SI>20 [4]. This is especially relevant because one of the *E. coli* strains tested is resistant to ciprofloxacin. In the same conditions, SI>2 was found even for hyper-biofilms producing clinical isolates from diabetic foot infections.

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Antimicrobial Photodynamic Therapy for Clinical Applications
Chair: Luis Arnaut

Photogeneration of Reactive Oxygen and Iodine Species by Modified Porphyrinoids: From Molecular Mechanisms to Advanced Preclinical Models in aPDT

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Keywords: antimicrobial photodynamic therapy (aPDT); porphyrinoids; reactive oxygen and iodine species; drug-resistant infections

The global threat of multidrug-resistant (MDR) bacteria has catalyzed the development of alternative therapeutic strategies. Antimicrobial photodynamic therapy (aPDT), which combines photosensitizers (PS), light, and oxygen, is emerging as a promising solution.[1-3]

In this study, we report the synthesis and evaluation of a series of porphyrin-based photosensitizers, including halogenated and imidazolyl derivatives, designed to enhance photogeneration of both reactive oxygen species (ROS) and reactive iodine species (RIS). Photophysical characterization confirmed efficient singlet oxygen production and, in some cases, iodide-mediated generation of cytotoxic iodine radicals. The most potent compounds demonstrated selective bacterial uptake and nanomolar-level activity against MDR strains under low light doses. Fluorescence Confocal and Scanning Electron Microscopy revealed extensive structural damage to bacterial envelopes post-aPDT, especially when PS were combined with membrane-disruptive agents such as verapamil or cinnamaldehyde. Advanced 3D keratinocyte infection models showed reduced bacterial colonization, while *in vivo* murine wound infection studies confirmed bacterial eradication and accelerated healing.[2-6]

Importantly, the optimized porphyrinoids displayed minimal toxicity towards mammalian tissues and high selectivity for pathogens. The dual ROS/RIS mechanism, validated by selective probe assays and histological analysis, offers a synergistic antimicrobial effect. These results support the use of structurally tailored porphyrinoids in the fight against drug-resistant infections and lay the groundwork for translational applications of aPDT in clinical contexts.

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Breaking down the resistance of bacteria to antibiotics and the treatment of pneumonia resistant using photoreaction

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Antibiotic resistance is one of the most serious challenges we are facing in the treatment of infections. Using basic mechanisms such as efflux pumps, enzyme production, membrane alteration, etc., antibiotic molecules become refractory. Well-controlled photodynamic effects allow the destruction of these molecules and return of susceptibility to the antibiotic. This demonstration will be presented in this presentation, as well as the various consequences that this has led to. Once resistance is broken, we must devise ways to combine photooxidation with the use of antibiotics. This will be demonstrated by the recent disinfection program for pharyngotonsillitis and by the present research for the treatment of antibiotic-resistant pneumonias.

Main references can be found at https://scholar.google.com/citations?user=bBN-6lQAAAAJ&hl=pt-BR

Antimicrobial Photodynamic Therapy for Clinical Applications
Chair: Luis Arnaut

Photodynamic therapy of acne and hidradenitis suppurativa

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Acne and hidradenitis suppurativa (HS) are chronic inflammatory disorders of the sebaceous glands and pilosebaceous units.

Topical conventional treatments are often ineffective and systemic treatments have several controindications, problems of toxicity and, again, may be no or only partially effective.

Topical treatment with aminolevulinic acid or methylaminolevulinic acid was found effective although pain was a major problem. In addition the need of a 2-3 hours occlusive medication was often a problem with young patients. In the case of HS, intralesional application of an ALA solution was reported as effective in the treatment of sinuses and cysts of severe HS, where topical PDT was not effective.

We have investigated efficacy, tolerability and safety of PDT with topical application of RLP068/Cl, a second-generation zinc(II)-phthalocyanine dye (Molteni Farma, Fi, I) followed after 30 minutes by red-light application in the treatment of severe papulo-pustular acne of the face and Hurley 2 and 3 HS of the groins and axillae.

In both pilot studies, RLP068/Cl-PDT was a safe, well-tolerated, and effective treatment option with a significative reduction of severity scores and no SAE.

Antimicrobial properties and safety profile of Protoporphyrin IX and pexiganan conjugate

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Keywords: Protoporphyrin IX, Antimicrobial peptides, pexiganan, Antimicrobial photodynamic inactivation, aPDI, *Pseudomonas aeruginosa.*

The emergence of multidrug-resistant (MDR) bacteria poses a global threat, as treating many infections has become increasingly difficult. This situation requires an immediate implementation of novel effective strategies as by 2050, deaths from drug-resistant infections are projected to surpass the annual number of cancer-related deaths recorded nowadays. Undoubtedly, antimicrobial photodynamic inactivation (aPDI) and antimicrobial peptides (AMPs) offer promising alternatives useful against MDR pathogens. The aPDI employs light-activated photosensitizer to generate reactive oxygen species (ROS), that damage microbial cells, while AMPs, natural components of innate immunity, disrupt cell membranes. Notably, combining aPDI with AMPs has been shown to enhance the photoinactivation of MDR pathogens, such as *Pseudomonas aeruginosa*, by increasing photosensitizer uptake and promoting membrane disruption [1]. Pexiganan, a 22-residues analog of a magainin derived from the African clawed frog, exhibits broad-spectrum antimicrobial activity and remains a focus of many clinical trials [2].

The aim of this study was to evaluate the efficacy of aPDI using protoporphyrin IX (PpIX) as a photosensitizer conjugated to the antimicrobial peptide Pexiganan. *P. aeruginosa* ATCC® 10145 was utilized as a model Gram-negative bacterial pathogen. Pexiganan was synthesized by solid-phase method using standard Fmoc chemistry on a polystyrene resin with a Rink Amide linker. Conjugation of PpIX to Pexiganan was achieved through direct coupling with N-terminal amino group, as well as through tetraglycine (-GGGG-) linker. Photodynamic treatment was carried out using blue light (λ_{max} 410 nm), with irradiation fluences of 5, 10, and 15 J/cm^2 and the constant irradiance of 15 mW/cm^2. Cytotoxicity and phototoxicity assays performed on mammalian cells indicated that the conjugates exhibited a toxicity profile comparable to that of PpIX alone, suggesting minimal added cytotoxicity from the AMP component. The results demonstrated that conjugation of porphyrin-based PS to selected AMPs can significantly potentiate the efficacy of aPDI, likely due to enhanced intracellular accumulation of the photosensitizer within bacterial cells. Among the tested constructs, the PpIX-GGGG-Pexiganan conjugate emerged as the most potent compound with superior antimicrobial performance.

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Shedding Light on Biofilms: Antimicrobial Efficacy of Low Irradiance Violet-Blue Light Exposure

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Keywords: Violet-blue light; Antimicrobial; Low irradiance; Biofilms

Background: Low irradiance violet-blue light (400-420 nm) has demonstrated application for safe, continuous, decontamination of occupied healthcare environments. The ability of low irradiance (<1 mW/cm^2) violet-blue light to reduce bacterial contaminants on clinical surfaces is well-documented; however antimicrobial efficacy of these low irradiance levels against biofilms – which have been detected on ~83% of hospital surfaces and represent a significant risk of pathogen transmission [2] – is broadly unknown, with most studies utilising higher irradiance levels (>10 mW/cm^2). **Aims:** The present study establishes the efficacy of low irradiance 405 nm violet-blue light for inactivation of bacterial biofilms of varying maturity attached to common clinical surfaces. **Methods:** To facilitate biofilm growth, 15 mm^2 coupons (PVC, stainless steel, glass and vinyl) were immersed in 10^6 CFU mL^{-1} *Staphylococcus aureus* suspensions for 1 h, before incubating in 3 mL sterile tryptone soya broth for either 4 h (monolayer biofilm) or 24 h (mature biofilm) at room temperature. Post-incubation, coupons were removed, washed in PBS, dried and exposed to 43.2 J/cm^2 violet-blue light (~0.5 mW/cm^2; 24 h); with identical control coupons exposed to ambient lighting. Surviving bacterial colonies were recovered and enumerated from the exposed side of coupons using a swabbing method. **Results:** Successful inactivation of both monolayer and mature biofilms was demonstrated (Figure 1), with reductions of 0.92-1.71 $\log_{10}$ CFU/coupon (66.6-96.7% inactivation) and 0.47-2.41 $\log_{10}$ CFU/coupon (61.2-99.4% inactivation) recorded, respectively; and trends across different surfaces established. **Conclusions**: This study successfully demonstrates the ability of violet-blue light, employed at levels safe for environmental decontamination, to inactivate bacterial biofilms of varying maturity, providing further evidence of the efficacy of 405 nm violet-blue light as a complementary decontamination technology for clinical infection prevention and control.

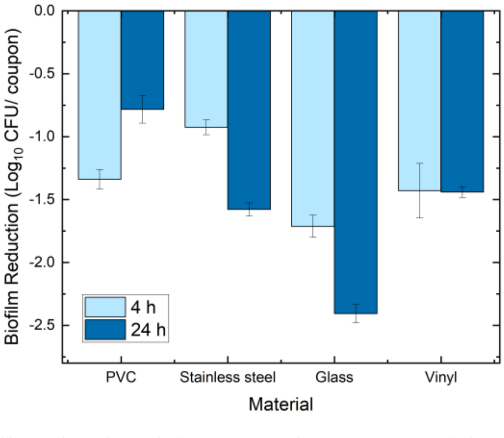

Figure 1. Reduction of *S. aureus* biofilms developed for 4 or 24 h on PVC, stainless steel, glass and vinyl coupons (15 mm^2) and exposed to 43.2 J/cm^2 405 nm light (~0.5 mW/cm^2; 24 h). (mean ± SEM; n=6). Biofilm counts on all 405-nm light exposed coupons were significantly lower ($P \leq 0.05$) than on ambient light exposed coupons.

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Antibacterial mechanisms of action of a new Ru(II) polypyridyl photosensitizer in Photodynamic Therapy

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Keywords:

Photodynamic therapy (PDT) consists of administrating a non-toxic photosensitizer (PS) followed by light irradiation to generate reactive oxygen species. Antimicrobial PDT (aPDT) has been suggested as a promising alternative to current antibiotic therapy (Youf et al., 2021). Amongst the considered PSs, ruthenium(II)-polypyridyl complexes are of great interest due to their already well-described stability, inertness, and synthetic tailorability (LeGall et al. 2018). A new ruthenium complex containing bidentate polypyridyl ligands was tested as a metal-based photosensitiser for aPDT applications. The efficiency and mechanisms of action were assessed on *Staphylococcus aureus* and *Escherichia coli.* The ruthenium complex showed good antibacterial activity against both microorganisms with > 7 $\log_{10}$ CFU inactivation. Confocal fluorescence microscopy experiments revealed cell internalization of the complex and clear localization within the cytoplasm of both *S. aureus* and *E. coli* bacterial species. The effect of the photodynamic treatment on cell viability was further explored studying the degradation of genomic DNA by agarose electrophoresis and nanopore long read sequencing revealing DNA damage due to the generation of reactive oxygen species. This study provides valuable insights for future research on mechanisms of action and design of photosensitizers (PSs) with enhanced antibacterial properties.

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Molybdenum cluster-based photosensitizers in antimicrobial photodynamic therapy and their applications

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Keywords: antimicrobials; photodynamic therapy; molybdenum clusters; photosensitizer

The growing threat of antibiotic resistance and biofilm-associated infections highlights the need for alternative antimicrobial strategies. This work explores the use of octahedral molybdenum clusters as photosensitizers in antimicrobial photodynamic therapy (aPDT).

These red-emitting luminophores and singlet oxygen photosensitizers can be efficiently excited across a broad spectral range—from UV to green light—to generate long-lived triplet excited states. The modular nature of their apical ligands allows for tailored physico-chemical properties to suit specific applications. In contrast to conventional organic dyes such as porphyrins, these complexes maintain strong luminescence in the solid state and show enhanced photostability due to their metallic core. These attributes make them promising candidates for the development of robust, high-performance luminescent materials.

The azide-based cluster $Na_2[Mo_6I_8(N_3)_6]$ exhibits strong phototoxic effects against Gram-positive pathogens (S. aureus, E. faecalis), including biofilm disruption and inhibition. It remains active in nutrient-rich media without inducing resistance. Moreover, several experiments exploring the induction of possible resistance have proven no change in the susceptibility to this compound.

Applications extend to wastewater treatment utilizing Mo_6/TiO_2 nanocomposites. In this case, enhanced electron transfer enhances ROS generation under daylight leading to the efficient destruction of pathogenic species. Additionally, hydrophobic ligand-modified clusters embedded in dermal emulsions showed selective phototoxicity against skin pathogens (*S. aureus, C. acnes*) while preserving commensal microbiota and demonstrating safety for topical use.

These findings position molybdenum clusters as promising tools for further resarch on light-activated antimicrobial therapies across medical and environmental domains.

This work was supported by grant no. A2_FPBT_2025_050 given by UCT Prague

Photochemistry of fluorescent proteins: from mechanistic studies to new fluorescence imaging methods

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Keywords: Fluorescent protein; Photoreaction; Mechanism; Fluorescence imaging

Fluorescent proteins (FPs) are extensively used in biological imaging as genetically-encoded fluorescent probes. However, FPs are not simple molecular beacons: they exhibit a variety of photoreactions whose mechanisms are only partially understood. These photoreactions are harmful when they compete with fluorescence, but they can also inspire the development of innovative imaging methods, as demonstrated for example by the emergence of super-resolution imaging. A better understanding of the photochemistry of FPs is essential to improve the performance of biological imaging.

Using the example of our work on Reversibly Switchable Fluorescent Proteins (RSFPs)[1-4] and on light-induced Reverse Intersystem Crossing (RISC) in EGFP,[5] I will show how detailed studies of the mechanisms of FP photoreactions, based in particular on time-resolved spectroscopy from the femtosecond to the millisecond scale, can lead to the proposal of new biological imaging methods.

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From Quantum Dots to Fluorescent Organic Nanoparticles: bright nanotools for biosensing

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Keywords: fluorescence, biosensors, biomarkers, bioimaging, FRET

The in situ and real-time detection of analytes in complex biological media demands robust, sensitive, and stable biosensors capable of signal amplification. Fluorescent nanoparticles (NPs) are promising candidates, offering exceptional brightness and photostability compared to traditional dyes.[1] These luminescent NPs fall into two main categories: intrinsically luminescent NPs, such as Quantum Dots (QDs), and doped NPs, which encapsulate dyes (e.g., lanthanides or organic fluorophores) within a matrix. For imaging and sensing applications, these NPs aim to achieve excellent brightness, enhanced photostability, and strong colloidal stability in water, outperforming conventional organic dyes.

Classical FRET (Förster resonance energy transfer) nanosensors typically involve a donor NP conjugated with bioreceptors that bind to a ligand labeled with an acceptor dye. While bioreceptors optimization has advanced detection limits and dynamic ranges, the roles of dye type and spatial configuration in these systems remain underexplored. This presentation addresses this gap by comparing organic fluorophores (e.g., Cy5, Texas Red) and inorganic NPs (QDs) as FRET donors or acceptors, identifying key molecular parameters that enhance sensor performance, and providing design guidelines for FRET-based assays and diagnostics.[2-3] Additionally, Fluorescent Organic Nanoparticles (dFONs) are introduced as metal-free alternatives to QDs, offering comparable brightness per volume. Obtained via nanoprecipitation of hydrophobic dyes, dFONs remain underutilized as biosensors due to limited functionalization strategies.[4] We demonstrate an innovative maleimide-thiol surface functionalization approach, enabling applications such as intracellular thiol sensing in the µM range5 and biotinylation for biomarker development. These advancements position dFONs as versatile, ultra-bright, and metal-free tools for next-generation diagnostics.

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Sense and Switch across the scales - GFP-like and Bacteriophytochromes in optical imaging from mice to cells

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Keywords: GFP-like proteins, bacteriophytochromes, photoswitching, sensors, fluorescence imaging, opto-/ photoacoustic

Photoswitching proteins of the green fluorescent protein (GFP)-like family have found application in super-resolution fluorescence imaging and beyond. Next to those, native photoswitching Bacteriophytochromes (BPhPs) have gained attention as labels in photo- / optoacoustic imaging in recent years. In both classes, photoswitching relies on an interplay of chromophore and protein matrix. Conversely, chimeric fluorescent sensors have long exploited the reversible influence of the protein matrix on the chromophore to generate fluorescence responses upon ligand binding. Combining switching and sensing might enable new applications for optical imaging. Beyond that such proteins integrating the influence of light control and ligand binding on chromophore photophysics can add new perspectives to the fascinating class of photoswitching proteins.

Fluorescent Protein Photobleaching: Spectroscopic Impact and Molecular Basis

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Keywords: Photobleaching, fluorescent proteins, fluorescence intensity and lifetime, mass spectrometry

Fluorescent proteins (FPs) are genetically encoded probes that play a pivotal role in biosciences, enabling the visualization and analysis of biological processes in living cells at the molecular scale. However, their utility is limited by photobleaching—the light-induced, irreversible loss of fluorescence—which constrains both the temporal and spatial resolution of imaging experiments. Various consequences of photobleaching have been observed [1-3], and several underlying mechanisms have been reported [4-5]. However, despite its significance, no systematic study of photobleaching has been conducted under comparable irradiation power and experimental conditions. This lack of standardization has made it difficult to directly compare photostability across different fluorescent proteins and to draw general conclusions about their photobleaching pathways.

In this study, we present a quantitative investigation of the photobleaching processes across a panel of FPs. Using in vitro approaches, we tracked spectroscopic changes—including absorption, steady-state fluorescence, and time-resolved fluorescence—under well-defined irradiation conditions. Photobleaching kinetics were assessed alongside the identification of photoproducts at the molecular level via mass spectrometry. Our results reveal multiple photophysical degradation pathways, clarify their relative contributions to photobleaching for each FP, and delineate their impact on fluorescence intensity and lifetime measurements. These insights advance our understanding of FP photostability and highlight the implications of photobleaching for fluorescence imaging applications.

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Dye-based Fluorescent Nanoparticles for Optogenetics

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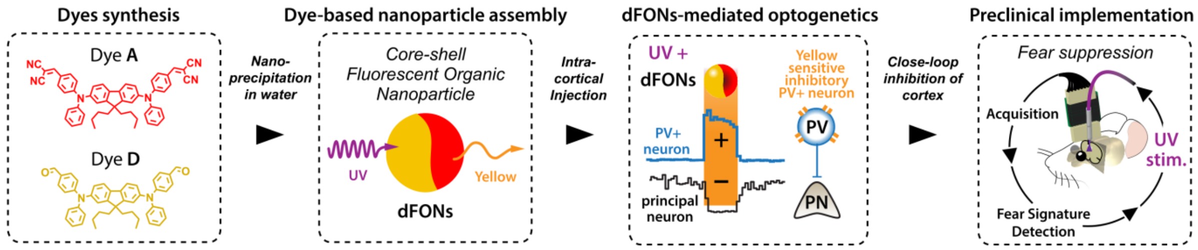
Keywords: Optogenetic; Fluorescence; Organic Nanoparticles; Neurostimulation

Dye-based fluorescent organic nanoparticles are a specific class of nanoparticles obtained by nanoprecipitation in water of pure dyes only.[1] While the photophysical and colloidal properties of the nanoparticles strongly depend on the nature of the aggregated dyes, their excellent brightness in the visible and in the near infrared make these nanoparticles a unique and versatile platform for in vivo application. These surfactant-, metal- and polymer-free fluorescent organic nanoparticles are bright and stable enough to be imaged at the single particle level in brain slices.[2]



Keeping this in mind, we evaluated the potential of these nanoparticles as promising nanotools for in vivo optogenetics applications[3] and the results are shown in this presentation. Their photophysical properties as well as their biocompatibility and their capacity to activate Chrimson opsin in vivo through fluorescence reabsorption process are demonstrated. Additionally, an illustrative example of employing these nanoparticles in fear reduction in mice through closed-loop stimulation is presented. Through an optogenetic methodology, the nanoparticles demonstrate an ability to selectively manipulate neurons implicated in the fear response and diminish the latter. Dye-based fluorescent organic nanoparticles represent a promising and innovative strategy for optogenetic applications, holding substantial potential in the domain of translational neuroscience. This work paves the way for novel therapeutic modalities for neurological and neuropsychiatric disorders.

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Engineering material-friendly proteins

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Keywords: Fluorescent proteins, materials, biohybrids, Circularly polarized luminescence.

Fluorescent proteins (FPs) have revolutionized biology as labels and sensors. Notably, only a few years after the cloning and engineering of GFP began, FPs successfully transitioned to synthetic materials for their characterization or for novel applications[1,2]. This trend has only accelerated since then[3,4]. Despite these advances, FPs face limitations in non-biological environments and lack resilience under unnatural stress conditions. To address this, we introduced a genetically encoded macro-oligomerization strategy that enhances FP protein-protein interactions through electrostatic control, applicable across various FPs[5]. These macro-oligomers remain stable for months in organic solvents and harsh conditions, making them suitable for integration into non-aqueous polymer-based materials (Fig 1). Our computer-based engineering approach also produced highly supercharged FPs (+22) that retain photoluminescence and thermal stability comparable to their native counterparts, forming self-assembled FP-apoferritin co-crystals within silicone[6].

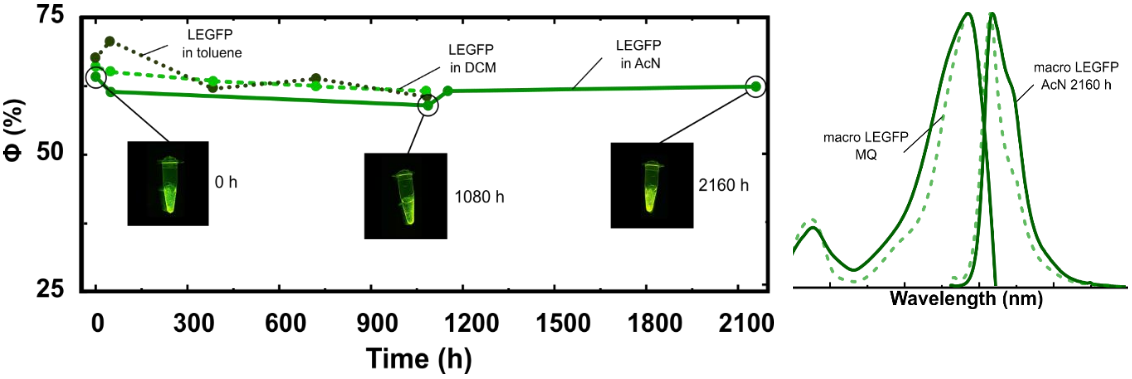

Figure 1, left: ϕ over time of LEGFP macro-oligomers in pure Acetonitrile (AcN), ….(DCM), and toluene; right: Excitation and emission spectra of LEGFP macro-oligomers in MQ water and after 2160 h in AcN

Additionally, we demonstrated that all FP classes can emit circularly polarized light (CPL), achieving unprecedented CPL brightness among organic emitters. Our findings reveal that CPL emission in β-barrel FPs is inherently linked to the chromophore's polarity, degrees of freedom, and exciton coupling[7]. Our findings suggest that CPL can be genetically controlled in the future.

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Selective Mitochondrial Targeting Using TAPY Vectors: From Cancer Cells to Patogenic Yeast

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Keywords: DLCs; imaging; cell, yeast.

Delocalized lipophilic cations (DLCs) are widely employed as mitochondrial targeting vectors, with triphenylphosphonium (TPP^+) being one of the most commonly used examples.[1]

In this study, we introduce TAPYs, triarylpyrylium-based cations, as a synthetically accessible and versatile alternative to TPP^+ for mitochondrial targeting. A set of six TAPYs derivatives bearing *para*-substituted aryl groups were synthesized, from commercially available aldehydes and ketones using a previously reported synthetic strategy.[2-3] To enable intracellular visualization and tracking, the $TAPY^+$ core was conjugated to a boron-dipyrromethene (BODIPY) fluorophore, generating fluorescent TAPY-BDP dyads.

Our results demonstrate that the $TAPY^+$ moiety is critical for mitochondrial localization, as confirmed by Confocal Laser Scanning Microscopy (CLSM). High Pearson's colocalization coefficients (PCC ≈ 0.9–1.0) were observed with Mitotracker Deep Red in cancer cell lines (MCF-7, A549, and HT-29), whereas normal and healthy cells (HEK-293 and HMEC-1) showed significantly lower values (PCC ≈ 0.4–0.7). Moreover, TAPY-BDP exhibited superior mitochondrial selectivity and fluorescence intensity compared to the classical TPP-BDP reference.[4] In addition to confirming the mitochondrial specificity of TAPY-BDP in human cancer cells, we evaluated their performance in pathogenic yeasts, including *Candida albicans* and *Pichia kudriavzevii*. High mitochondrial colocalization was observed by CLSM, and flow cytometry confirmed efficient cellular uptake and mitochondrial accumulation. These results were benchmarked against traditional mitochondrial vectors such as TPP^+.

In summary, we propose $TAPY^+$ cations as promising alternatives to conventional mitochondrial vectors. Their synthetic flexibility and enhanced targeting capacity in both human cancer cells and pathogenic fungi make them attractive candidates for applications in mitochondrial bioimaging and targeted therapies.

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Ultrafast Spectroscopy of High-Light Induced Proteins

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Keywords: photosynthesis, photoprotection, high-light induced proteins

High-light induced proteins (Hlips) are small membrane proteins evolutionary related to the LHC antennae of algae and plants. Hlips are found in cyanobacteria, but similar proteins, such as light-harvesting-like (LIL) or early light-induced proteins (ELIPs) occur also in plants. All these proteins play an important role in photoprotection as the chlorophylls bound to these proteins are efficiently quenched as evidenced by a significant decrease of chlorophyll fluorescence. Ultrafast spectroscopy identified the quenching mechanism both in Hlips [1] and LIL/ELIPs [2] as efficient energy transfer between excited Chl-a and the S_1 state of the carotenoid. The energy transfer occurs on a time scale of a few picoseconds, making these proteins ideal quenchers. Recently, we have expanded our studies to other Hlips binding different carotenoids. An interesting member of the Hlip family is HliZ5 protein from *Legtolyngbya*, which, in contrast to other Hlips, is a three-helix protein. HliZ5 binds the keto-carotenoid echinenone in a 2:1 Chl-a:Car ratio. Ultrafast spectroscopy data show that excitation of Chl-a generates the S1 state of echinenone within a few picoseconds, identifying the HliZ5 as efficient quencher employing quenching mechanism identical to those reported for other Hlips and LIL/ELIP proteins. Data analysis demonstrated that in HliZ5 the 5.6 ps lifetime of the quencher (the S_1 state of echinenone) is shorter than the Chl-a to echinenone energy transfer time (8 ps). Our data provides evidence that quenching in Hlips is a robust process which operates in very similar way regardless of the bound carotenoid and/or protein structure. All these quenching proteins exhibit significantly red-shifted carotenoid absorption, which is a marker of the quenching condition and a necessary condition to observe efficient quenching. Comparison of cryo-EM structural data on Hlips in complex with chlorophyll synthase with known structures of other LHCII-type proteins indicates that the key structural feature leading to the efficient quenching is likely a specific configuration of the terminal ring of the carotenoid quencher. This carotenoid configuration decreases the energy of the energy acceptor state (carotenoid S_1 state), making the energy transfer from Chl-a to carotenoid energetically favorable.

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Development Multiscale of Time-Resolved Circular Dichroism for Investigating Conformational Changes of Biomolecules

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Keywords: Circular dichroism, DNA, folding, ultrafast spectroscopy

Circular dichroism (CD) spectroscopy, which measures the differential absorbance of left- and right-hand circularly polarized light, is a powerful tool for analyzing biomolecular secondary structures in solution at equilibrium. By combining pump-probe methods with CD spectroscopy, it becomes possible to track conformational and electronic changes in proteins and DNA over a wide range of time scales. However, time-resolved CD measurements remain difficult, particularly at femto-picosecond timescales, due to weak signals prone to pump-induced artifacts [1]. In recent years, we have developed several experimental set-ups for measuring light-induced CD changes ranging from a few hundred femtoseconds to seconds [2, 3]. This presentation will discuss their principles and applications, focusing on our recent research on the photoinduced conformational dynamics in particular DNA structures, the G-quadruplexes [4].

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Ultrafast multi-exciton spectroscopy tracks excitation energy transport through molecular photosystems

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Keywords: Ultrafast spectroscopy, Excitation transport, Photosynthesis

Electronic excitation transport lies at the core of photosystem functionality. Tracking the excitation through a complex spatial-energetic landscape, at femtosecond to nanosecond timescales over nanometer to micrometer distances is a challenging task typically attempted by a combination of ultrafast spectroscopy techniques. Bridging the ultrafast local relaxation with long-range diffusion remains an open challenge both for theory and experiment [1].

In this contribution, I will discuss a straightforward modification of ultrafast spectroscopy such as transient absorption that systematically disentangles dynamics of single, two and more excitations by decomposition of the signal into increasingly nonlinear contributions (see Figure below) [2,3]. Analyzed together, the multi-exciton signals allow to track the excitation dynamics continuously from the initial wavelike dynamics of individual excitations to the long-range diffusive transport visualized by multi-particle interaction. The method is universal, applicable to different ultrafast spectroscopy techniques and any samples including photosynthetic complexes [2,4].

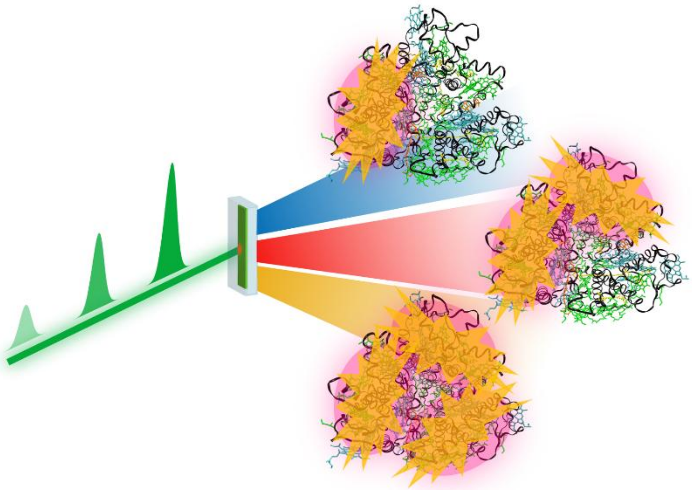

Figure Using a sequence of defined pulse intensities, our approach can decompose transient spectroscopy signals into those tracking increasing number of excitations (here shown in LHCII photosynthetic complex of plants).

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Spatio-Temporal Imaging of Exciton Energy Transfer below Sunlight Level

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We can learn of lot of the light harvesting design of nature: how does nature dynamically re-organize the membrane architecture, its packing, order, diffusion, on light stress?; which pathways are taken to charge separation?; what is the role of fluctuations, coherences, colour and vibrations?

I will present advances in the development of nanoscale ultrafast imaging tools [1-4], particularly a novel spatiotemporal microscopy technique that exploits structured excitation in order to dramatically decrease the excitation intensity, up to 10,000-fold when compared with previously reported spatiotemporal photoluminescence microscopy experiments. Indeed, nanometer-scale energy transport was directly observed for the first time in both space and time in a printed monolayer of the light-harvesting complex 2 from purple bacteria.

Secondly, I will expand into spatio-temporal-spectroscopic methods detecting charges, essentially photocurrent microscopy, to resolve the transfer from the light-harvesting antenna complexes towards the reaction centres, applied to plant PS1[5-8]

This work is part of the project ERC Advanced Grant 101054846 *FastTrack*.

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Excited-state dynamics of neorhodopsin, an bistable microbial rhodopsin: retinal to retinal energy transfer and nonlinear effects

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Keywords: rhodopsin; ultrafast spectroscopy; excitation energy transfer; nonlinear dynamics

Neorhodopsin (NeoR) is a newly discovered fungal bistable rhodopsin that reversibly photoswitches between UV- and near-IR absorbing states denoted NeoR$_{367}$ and NeoR$_{690}$, respectively. NeoR$_{367}$ represents a deprotonated retinal Schiff base (RSB), while NeoR$_{690}$ represents a protonated RSB. Cryo-EM studies indicate that NeoR forms homodimers with 29 Å center-to-center distance between the retinal chromophores. UV excitation of NeoR$_{367}$ takes place to an optically allowed S3 state of $1B_u^+$ symmetry, which rapidly converts to a low-lying optically forbidden S1 state of $2A_g^-$ symmetry in 39 fs, followed by a multiexponential decay to the ground state on the 1 - 100 ps timescale. A theoretically predicted np* (S2) state does not get populated in any appreciable transient concentration during the excited-state relaxation cascade. We observe an intra-dimer retinal to retinal excitation energy transfer (EET) process from the NeoR$_{367}$ S1 state to NeoR$_{690}$, in competition with photoproduct formation. To quantitatively assess the EET mechanism and rate, we experimentally addressed and modeled the EET process under varying NeoR$_{367}$-NeoR$_{690}$ photoequilibrium conditions and determined the EET rate at $(200\ \text{ps})^{-1}$. The NeoR$_{367}$ S1 state shows a weak stimulated emission band in the near-IR around 700 nm, which may result from mixing with an intramolecular charge-transfer (ICT) state, enhancing the transition dipole moment of the S1-S0 transition and possibly facilitating the EET process. We suggest that EET may bear general relevance to the function of bistable multi-wavelength rhodopsin oligomers.

The NeoR$_{690}$ to NeoR$_{367}$ photoreaction is known to exhibit a very low quantum yield of approximately 0.1%. We characterized the inefficient photochemistry of NeoR$_{690}$ by means of highly sensitive multi-timescale TA spectroscopy. At high excitation conditions, we observed a singlet-singlet annihilation process ascribed to EET among RSB chromophores within the NeoR dimer at 5 ps and 60 ps timescales. We find that under annilation-free conditions the excited state decays by a single lifetime of 1.3 ns. Under high power conditions, a photoproduct P700 is formed at a yield of 1%. P700 evolves to a secondary product P750 at a yield of 10%, resulting in a net product yield of 0.1%. Despite the matching quantum yield, we suggest that P700 and P750 do not correspond to genuine NeoR$_{690}$ photoproducts and instead result from ionized species due to excessive excitation energy.

Tracking Energy Transport in Photosynthetic Systems: Does Nature Know Better?

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Keywords: Exciton, Transport, LH2, Porphyrins

This work delves into the realm of energy transfer within natural photosynthetic systems, aiming to address the growing 21st-century demand for sustainable bioenergetics solutions. Understanding how energy is transported efficiently in nature over long distances is a critical factor for enhancing light-energy conversion in artificial systems. While considerable progress has been made in elucidating energy transfer within individual photosynthetic antenna proteins, the dynamics of inter-protein transfer remain poorly understood due to the complex three-dimensional organization of these networks.

Here, I present the first direct measurements of exciton diffusion in photosynthetic systems, demonstrating the critical role of intermolecular distance in regulating transport by systematically varying the packing density. Crucially, these transport measurements are conducted under sub-solar excitation intensities using a novel technique developed in our group in 2024 [1] — StrEET (Structured Excitation Energy Transfer) — which enables high signal-to-noise measurements at excitation fluences up to 10,000 times lower than any previously reported method [2].

This study focuses on two model systems: the LH2 antenna complex from *Rhodobacter (Rba.) sphaeroides* purple bacteria, and porphyrin molecules, which serve as a model for BChl-c found in green sulfur bacteria. Densely packed porphyrin films exhibit diffusion coefficients ($D = 0.012\ \text{cm}^2/\text{s}$) comparable to state-of-the-art organic photovoltaics, with diffusion lengths extending to 55 nm. Similarly, LH2 networks at natural packing densities achieve even higher diffusion coefficients ($D = 0.019\ \text{cm}^2/\text{s}$), enabling exciton transport across 24 nm and spanning approximately 42 complexes — values consistent with native membrane architectures.

These findings offer new insights into the relationship between molecular organization and excitonic transport, showing that increased packing density enhances diffusion coefficients while reducing exciton lifetimes. As a result, the optimal diffusion length emerges as a trade-off between these competing effects, offering guiding principles for designing artificial light-harvesting systems inspired by natural processes and advancing our understanding of nature.

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Real-time tracking of ground- and excited-state responses of photoactive molecules to a change of microenvironment, from femtoseconds to milliseconds

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Keywords: Ultrafast spectroscopy; pH jump;

Light triggers fundamental biological processes in nearly all living organisms, by promoting (multi)chromophoric biomolecular systems to an excited state, which activates their function. The ability of these photoactive systems to absorb and use solar energy is often controlled by their microenvironment, which modulates their structural and energetic landscape. Several stimuli-responsive photoactive materials are designed based on this principle.

Understanding how photoactive molecular systems respond to light and environment requires tracking changes at both the electronically excited- and ground-state levels, with ultrafast resolution and across a broad range of timescales. Indeed, while light-induced excited-state dynamics involve processes as rapid as a few femtoseconds, the structural and electrostatic responses to changes of microenvironment occur at timescales orders of magnitude slower, up to the millisecond and beyond.
We demonstrate that this investigation can be enabled by a novel and universal three-pulse ultrafast spectroscopy method that: (i) induces controlled changes of environment, specifically pH, and (ii) probes photoactive systems' rapid responses in the ground and excited state from femtoseconds to milliseconds. Controlling changes of microenvironment and – simultaneously - bridging the ultrafast timescales of excited-state dynamics with the slower ones of environmental and conformational changes, is key to advancing our understanding of how living organisms and artificial materials regulate the interplay between light, environment, and structure in light-induced molecular processes.

Living Materials for Solar Energy Conversion from Photosynthetic Microorganisms and Biopolymers

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Keywords: solar energy; biohybrid; microalgae; biomaterials

The combination of photosynthetic microorganisms with tailored molecules and polymers to engineer biohybrid materials for solar energy conversion represents a promising and innovative frontier.[1] We have devised multiple strategies to improve light harvesting and carbon capture efficiency in photosynthetic microorganisms,[2] along with developing biopolymer-based interfaces that integrate photosynthetic cells or active photoenzymes with electrodes for photocurrent generation.[3,4]

We have successfully interfaced metabolically versatile purple photosynthetic bacteria with electrodes using polydopamine as a bio-adhesive layer. This configuration preserves cellular activity while enabling efficient transfer of photo-induced electrons, resulting in stable and sustained photocurrent generation.[5] In a complementary approach, we demonstrated that diatom microalgae can be directly immobilized on ITO surfaces to fabricate living electrodes. These biohybrid systems exhibit remarkable resilience, enduring multiple desiccation cycles and maintaining photocurrent production for up to 15 days in photoelectrochemical cells.[6] Moreover, naturally nanostructured biosilica extracted from these same microalgae can be used for the design of advanced smart materials; for example, to create magnetic shuttles to bear and spatially transport living neurons inside a microchip-like device.[7]

Overall, our biohybrid systems exhibit remarkable flexibility and sustainability, laying the foundation for a new generation of living technologies aimed at clean energy production and environmental remediation.

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Light management by photosynthetic micro-organisms
Chairs: Angela Falciatore & Gianluca Farinola

Microscale imaging sheds light on species-specific strategies for photo-regulation and photo-acclimation of microphytobenthic diatoms

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Keywords: Diatoms; Microphytobenthos; Photo-regulation; Movement

Intertidal microphytobenthic (MPB) biofilms are hotspots of coastal primary production [1], primarily driven by pennate diatoms that regulate light stress through non-photochemical quenching (NPQ) and vertical migration. In motile (epipelic) diatoms, migration is the dominant photoregulatory mechanism [2], enabling them to avoid high irradiance and display traits of low-light acclimation, such as reduced NPQ capacity compared to non-motile (epipsammic) forms [3]. However, many previous studies have focused on low-light acclimated monocultures (e.g. [4]), which do not reflect the complex and dynamic conditions of natural biofilms. In this study, we combined variable chlorophyll fluorescence imaging, fluorescent cell labelling in sediment cores, and scanning electron microscopy to investigate species-specific NPQ responses and migratory behaviours of four epipelic diatom species from a natural MPB community. These species exhibited a broad range of NPQ capacities—greater than those previously reported for epipsammic diatoms—suggesting functional diversity in their light response strategies. This diversity may facilitate their coexistence within tightly packed, vertically stratified light niches in MPB biofilms. Moreover, we demonstrate that diatom cell orientation within the sediment matrix is light-dependent: under high irradiance, cells tend to align more perpendicularly to the surface, while under low light, a parallel orientation is favoured. This behaviour suggests a novel photoregulatory strategy based on modulating the cellular light absorption cross-section. Together, these findings emphasise the need to consider species-specific photo-behaviour and spatial orientation when investigating MPB community function and light-use dynamics.

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Photobiological Hydrogen Production Using Photosynthetic Microorganisms

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Keywords: biohydrogen; photosynthetic bacteria; clean energy

Hydrogen is widely regarded as the most promising green fuel among clean and renewable energy sources, owing to its high energy density, conversion efficiency, and sustainability. Among various production methods, biological hydrogen generation is the most environmentally friendly, with photosynthetic microorganisms offering the greenest route [1]. These bacteria can convert organic acids into biohydrogen, and their growth can be supported by biodegradable industrial byproducts rich in carbohydrates—such as brewery and coffee wastes—serving as low-cost, sustainable feedstocks. The photosynthetic bacterium *Rhodobacter sphaeroides* was selected for its exceptional metabolic flexibility and its ability to export electrons via extracellular electron transfer (EET). Both its growth and photoinduced electron transfer processes—anodic and cathodic—can be fine-tuned to optimize hydrogen production. To further enhance EET efficiency and system stability, *R. sphaeroides* cells were encapsulated with polydopamine (PDA) [2, 3], a bioinspired conductive polymer. PDA acts as an interface mediator, improving contact between cells and the electrode surface while preserving cellular viability and activity.

Finally, coupling this strategy with the use of waste-derived substrates offers a novel and sustainable approach to industrial waste valorization, enabling clean energy production through biohydrogen.

Fundings:
This research was funded by the European Union – NextGeneration EU from the Italian Ministry of Environment and Energy Security POR H2 AdP MMES/ENEA with involvement of CNR and RSE, PNRR – Mission.

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Biocompatible Strategies for Microalgae-based Bio-photoanode Fabrication and Electron Extraction

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Keywords: photocurrent; diatoms; polydopamine; biopolymer.

Biophotovoltaic (BPV) devices are becoming a serious alternative to traditional silicon-based photovoltaic panels, thanks to several potential benefits like biodegradability, recyclability and durability. The use of intact photosynthetic microorganisms over isolated photoactive proteins carries significant advantages, as demonstrated by the successful implementation of cyanobacteria, green and even brown microalgae (diatoms) in BPV devices.[1] This last group has been largely underrepresented in the field, with only some remarkable works reported.[2] Diatoms can provide significant photocurrent intensities, while demonstrating resistance to stressors such as desiccation, which comes as an advantage when it comes to processing them into a living bioanode.[3] Moreover, diatoms hold the unique characteristic of bearing nanostructured biosilica microscopic shells (frustule), that can be functionalized with active molecules or polymers. With one of the main challenges in BPV design being electrical communication between the microorganism's metabolism and the electrode surface, different strategies have been developed, based on direct electron transfer, or the use of soluble electrochemical mediators.[4] The use of biocompatible, conductive polymers could increase the electron flow, while providing a suitable scaffold to an electrogenic community of microorganisms.[5] This work aims to study the photocurrent extraction from different species of green microalgae and diatoms, starting with an evaluation of their electrogenicity and resistance to the extraction process; followed by an exploration of several bio-inspired mediators and organic polymers, to bridge the photosynthetic apparatus and the BPV cell. Cyclic voltammetry and chronoamperometry measurements have been performed inside a three-electrode cell. Their interaction with different soluble mediators and polymers has been thoroughly studied. Up to this point, we have standardized a method to produce robust microalgae-based bio-photoanodes, resistant to desiccation and we have identified promising biocompatible approaches to ease the transfer of electrons between the biotic and abiotic components of a BPV cell.

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Bridging Diamond and Cyanobacteria: the Role of Polydopamine in Photocurrent Generation

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Keywords: Boron-doped diamond (BDD); Biophotovoltaics; Photoelectrochemistry; Green energy; Limnospira; Polydopamine (PDA)

Harnessing the natural photosynthetic capabilities of microorganisms through engineered biohybrid interfaces offers an exciting avenue for green energy production for Earth and **space applications**. However, to operate efficiently under the harsh conditions of space, material compatibility and resilience to ionizing radiation are paramount.
In this study, we introduce a **novel biohybrid electrode**, integrating the radioresistant cyanobacterium ***Limnospira indica*** **PCC 8005 (1,2)** with **boron-doped diamond (BDD)**, a chemical and radiation-tolerant wide bandgap semiconductor. The interface between both materials is enhanced using **polydopamine (PDA)**, a biocompatible and redox-active polymer inspired by mussel adhesion proteins.

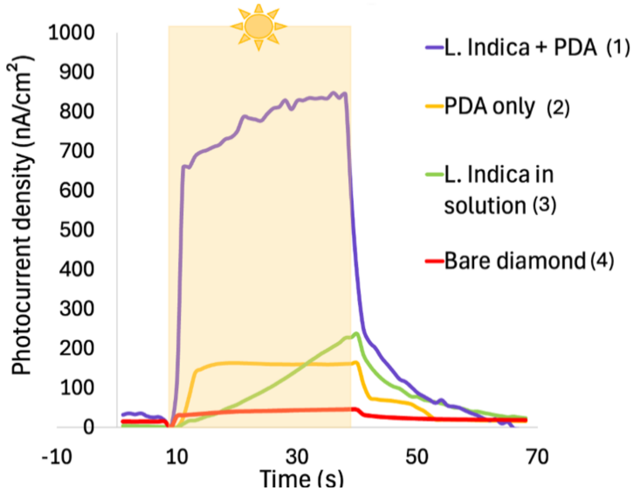

Figure 2: Photocurrent densities of different systems upon illumination with white light.

Short outline: the bio-hybrid electrode was fabricated by electropolymerizing PDA directly onto BDD in the presence of *L. indica,* by cycling the applied potential between -0.5 and 0.5 V for 50 cycles, leading to the formation of a stable, light-responsive layer. **While neither PDA (2) nor *L. indica* in solution alone (3) produced significant photocurrent upon illumination with white light, their combination (1) led to a four-fold increase, reaching 0.75 µA/cm²** (see figure). This photocurrent is attributed to synergistic direct and mediated electron transfer (DET and MET) pathways, facilitated by the PDA network, as supported by chronoamperometric measurements using a 3.5 kD dialysis membrane between the biological layer and the diamond electrode to block DET. Conceptualization of the system and findings will be discussed.

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Acknowledgements: This research is funded by The Research Foundation – Flanders (FWO) Frank De Winne Fellowship (1SF1423N) and The European Space Agency (ESA) project No. 4000137808 (OSIP Idea ID I-2021-00820).

Photosensitivity – The Role of Photodiagnostics

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Keywords: Photodermatoses, Photosensitivity, Photodiagnostics, Phototesting

A diagnosis of abnormal photosensitivity may be obvious on clinical assessment alone, without the need for photodiagnostic investigations, such as with classical polymorphic light eruption. However, photosensitivity is frequently not considered as a possibility, either by the patient or their physician, and this can lead to delayed or missed diagnoses. Accurate diagnosis is essential to advise patients and physicians regarding appropriate management, and photosensitivity diseases are a diverse group of conditions, with heterogeneity both in clinical presentations and photodiagnostic findings.

Narrow waveband ultraviolet (UV) and visible light phototesting, 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 disease. Additional photodiagnostic investigations, including broader band iterative UV provocation testing, patch and photopatch testing, 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[1]. This highlighted the diversity of photosensitivity diseases and the challenges of photodiagnosis and attempted to initiate a consensus approach between Centres and identify research priorities, including defining normal range UV-erythemal responses in people with skin of colour. Furthermore, the presence and nature of photodiagnostic services worldwide is unknown and this is currently being investigated through the Global Assessment of Photodiagnostic Services (GAPS) project.

Raising awareness of the photosensitivity conditions and of the key components of photodiagnostic investigations and services is important to allow patients to be accurately diagnosed and managed. This is essential as the major adverse impact of significant 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 the accessibility, ease and efficiency of such photodiagnostic services, with a view to improving the lives of patients with photodermatoses.

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Phototherapy of atopic dermatitis

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Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disorder characterized by intense pruritus and a significant impact on quality of life. While topical and systemic treatments remain the cornerstone of management, phototherapy has emerged as a valuable, evidence-based therapeutic option, particularly for moderate-to-severe and refractory cases. This presentation will provide an updated overview of the mechanisms, efficacy, and safety of various phototherapeutic modalities in AD, including narrowband UVB and UVA1. Special emphasis will be placed on patient selection, treatment protocols, long-term outcomes, and integration into personalized therapeutic strategies. Recent insights into the immunomodulatory effects of phototherapy and its role in the era of biologics will also be discussed. Phototherapy continues to be a clinically relevant, cost-effective, and well-tolerated approach in the multidisciplinary management of atopic dermatitis.

Photoprotection in the photosensitivity diseases”

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Keywords: photosensitivy disorders, photoprotection.

EXTENDED ABSTRACT

Background: Photosensitivity disorders are a diverse group of conditions triggered or worsened by exposure to ultraviolet (UV) and visible light. These include immunologically mediated diseases (e.g., polymorphic light eruption, actinic prurigo), metabolic (e.g., porphyrias), genodermatoses (e.g., xeroderma pigmentosum), and photoaggravated dermatoses such as lupus or eczema. Patients often experience significant impairment in quality of life.

Objectives: To review current strategies in the photoprotection and clinical management of photosensitive disorders, with emphasis on the efficacy of different photoprotective approaches and the role of emerging therapies.

Methods: Data from multiple in vitro and clinical studies were analyzed, evaluating sunscreens, fabrics, hats, antioxidants, systemic agents (e.g., *Polypodium leucotomos*, afamelanotide), DNA-repair enzymes, and behavioral interventions. Protection was assessed in terms of UVR/visible/infrared attenuation, clinical outcomes (e.g., PLE symptom reduction), and patient adherence.

Results: High-UPF clothing and broad-spectrum sunscreens with critical wavelength >380 nm are essential. Some patients benefit from tinted formulations or products enriched with antioxidants or DNA-repair enzymes. Agents such as ectoin, calcipotriol, and *Polypodium leucotomos* showed added value in PLE. Dupilumab and omalizumab are promising systemic therapies for selected photodermatoses. Psychological interventions (e.g., XPAND program) significantly improved photoprotection behaviors in xeroderma pigmentosum. Infrared radiation may also play a key role in photodermatosis pathogenesis, suggesting the need for broader protection strategies.

Conclusions: Management of photosensitivity disorders requires a multifactorial approach: physical protection, tailored topical formulations, systemic photoprotectors, and behavioral interventions. Ongoing research on new filters, antioxidants, and biologics is expanding therapeutic options and improving quality of life for affected patients.

Exploring the Repurposing of ExpoDose® for Disease Management of Photodermatoses

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Keywords: Photodermatoses, photomedicine, photosensitivity

Introduction: Photodermatoses, including conditions such as Solar Urticaria and Chronic Actinic Dermatitis, are characterized by heightened skin sensitivity to both outdoor and, occasionally, indoor light. This sensitivity may stem from altered immune reactions, genetic factors, side effects of medications or over-the-counter remedies, or from unknown causes [1]. The symptoms, which can include intense pain, itching, and rashes lasting several days or more, can severely impact the quality of life and mental health of sufferers, with rates of depression and anxiety approximately double that of the general population [2]. Disease management typically involves sunlight avoidance and photoprotection. However, there are few tools available that provide real-time information on accumulated light exposure and its potential to trigger photosensitivity.

Method: Following discussions with photosensitive patients at the Scottish Photobiology Service, we explored the potential of repurposing the research tool ExpoDose® (Figure 1, www.expodose.com, siHealth Ltd) to assist in managing light sensitivity conditions, as part of clinical service development. ExpoDose® monitors personal solar exposure via a smartphone app, utilizing GPS location and satellite data to calculate sunlight incident on the user. The project, named APPETISER (**A**ssisting **P**hotosensitive **P**atients **E**xcel **T**hrough **I**nformation on **S**un **E**xposure: a **R**ealism Study), aims to investigate the technical, practical, and experiential aspects of this technology with a group of light-sensitive patients. Patients will provide feedback on their engagement with the technology, its impact on managing their photosensitivity, and the perceived value of the tool.

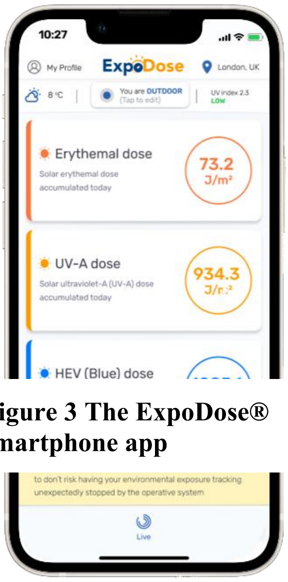

Figure 3 The ExpoDose® smartphone app

Results: The main phase of the project commenced in April 2025 and will conclude in July 2025. Initial feedback indicates that 80% of patients use the ExpoDose® app daily, with the same percentage finding the app easy to use. Early-stage feedback also shows that 40% of users are satisfied with the app's performance, while 30% are dissatisfied, potentially due to early technical issues, which affected 70% of users.

Conclusions: Initial experiences of photosensitive patients with the ExpoDose® app are varied. Technical difficulties have influenced perceptions, but encouragingly, a significant percentage of users are satisfied with the app's performance. Subsequent questionnaires will aim to determine whether ExpoDose® influences the management of photosensitivity conditions and the value patients place on the technology.

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A two-year follow-up of EPP patients during afamelanotide treatment indicates increases in the quality of life, time spent outside and demasking of pre-existing pigmented lesions

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Keywords: Erythropoietic protoporphyria, afamelanotide, inflammation, pigmented lesions

EXTENDED ABSTRACT

Erythropoietic protoporphyria (EPP) is an inherited porphyria caused by the defective activity of ferrochelatase (FECH), resulting in painful and debilitating phototoxicity In recent years, afamelanotide, a synthetic alpha-melanocyte stimulating hormone (α-MSH) analogue has been successfully used to alleviate symptoms and increase patients' quality of life. Since α-MSH stimulates melanocytes through engagement of its cognate melanocortin 1 receptor (MC1R), we were interested in the long-term dynamic change in the frequency as well as distribution of demarcated pigmented skin lesions including nevi. In the present study, we monitored the quality of life, time spent outside, phototoxicity events and the number of demarcated pigmented lesions as well as their distribution in 108 EPP patients over a period of 24 months. At baseline, the distribution of pigmented lesions was significantly associated with subjects` increased age, Fitzpatrick skin type and the measured body sites, but not gender. An increase in the number of demarcated pigmented lesions was apparent after the first year of treatment, but plateaued during the second year, indicating a possible demasking effect of pre-existing demarcated pigmented lesions such as nevi during afamelanotide treatment. Body site-specific increases were observed in sun-exposed areas, as expected. Notably, no malignant pigmented skin lesions were observed. Taken together, the present study underscores the benefit of afamelanotide treatment in EPP patients and points toward a desmasking effect of MC1R signaling for pre-existing pigmented lesions but does not indicate *de novo* nevogenesis.

A New Photoprotective Technology:

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Keywords: Photoprotection; SPF; Photostability; *Aspalathus linearis*; *Polypodium leucotomos*.

We are currently facing the challenge of designing alternative photoprotective strategies adjusted to cover the needs observed by the clinicians, while keeping in mind the optimization of the general population´s adherence and safety [1]. This has accelerated the search for new photoprotective ingredients, and natural extracts represent the most promising and attractive source [2].

Aiming to identify, characterize, and develop new photoprotective ingredients, we present here Aspa-Fernblock®, a novel botanical technology derived from the combination of two different *Aspalathus linearis* extracts and a specific extract from *Polypodium leucotomos*. First, we employed a cellular model to address fibroblast survival rates after UV exposition, observing a synergistic photoprotective effect in treatments with a determined combination of both *A. linearis* extracts [3]. Considering the presence of UV-absorbing metabolites (flavonoids, polyphenolic compounds….), and working towards the practical application of these extracts in photoprotection, we analyzed the UV filtering potential of the *A. linearis* extracts when included in topical formulations (sunscreens), by *in vitro* assays following ISO 24443:2022. Interestingly, the extracts provided a booster effect in SPF and UVAPF when combined with standard filters for UVB and UVA, increasing protection factors over 50% when compared to the results of the standard filters alone. Moreover, the photostability of avobenzone and octinoxate was significantly increased when combined with *A. linearis* extracts after a solar simulated dose corresponding to 5.7 minimal erythemal doses for a phototype II. Finally, *A. linearis* extracts were included in a commercial sunscreen formulation, and the SPF was measured under *in vivo* assays following ISO 24444:2021, showing an increase of 14,6% of SPF. Additionally, employing the mentioned cellular model addressing fibroblast survival, a complementary photoprotective effect was observed when testing these two *A. linearis* extracts in larger combinations with a specific extract from *Polypodium leucotomos* [3], supporting its jointed use as Aspa-Fernblock®.

Altogether, these results represent a solid scientific grounding for a natural photoprotective ingredient that would facilitate the activity of the skin´s endogenous protective mechanisms, but also possesses interesting filtering properties, being able to increase the SPF values of complex formulations and their photostability. This encourages the inclusion of Aspa-Fernblock® as a proper biological filter in future alternative photoprotective strategies, addressing important concerns in the field.

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Changes in ultraviolet B radiation-induced DNA damage and erythema after oral nicotinamide and polypodium leucotomas in healthy volunteers – an intraindividual controlled trial

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Keywords: Photoprotection, ultraviolet radiation B, nicotinamide, polypodium leucotomas

Introduction & Objectives:
Skin cancer, the most prevalent malignancy worldwide, is primarily caused by ultraviolet radiation (UVR), which damages lipids, proteins, and DNA, leading to erythema, premature aging, and consequently skin cancer. The rising incidence of keratinocyte carcinomas emphasizes the need for improved photoprotective measures. Systemic photoprotection is increasingly utilized. This study evaluates the photoprotective effects of nicotinamide (NAM) and polypodium leucotomas (PL).

Materials & Methods:
This intraindividual controlled trial included 50 healthy, skin phototype I-III, participants, divided into two groups receiving either NAM (2000 mg daily) or PL (480 mg daily) for 30 days. The photoprotective effects of these substances were assessed using a narrowband UVB (TL01) light source. The minimal erythema dose (MED) was evaluated, along with cyclobutane pyrimidine dimers (CPDs) in urine and in nuclear DNA of skin biopsies before and after treatment.

Results:
Of the 50 participants, 47 completed the study. The NAM group showed no significant change in MED after treatment ($p=0.533$), with a median MED of 0.41 J/cm² pre-NAM and 0.47 J/cm² post-NAM. Conversely, the PL group exhibited a significant increase in MED ($p=0.00018$), with a median MED of 0.41 J/cm² pre-PL and 0.53 J/cm² post-PL. No significant difference in CPDs was observed between pre- and post-treatment in urine samples, NAM ($p=0.85$) and PL ($p=0.68$) groups after creatinine correction and likewise in the skin biopsies for both groups ($p>0.05$).

Conclusion:
PL reduced skin redness following UVB exposure but did not protect against DNA damage. NAM had no effect on inflammation of the skin after UVB exposure and did not protect against DNA damage. These findings suggest that while PL decreases redness upon UVB exposure, it does not prevent DNA damage and, consequently, skin cancer. Further studies are necessary to investigate reproducibility and with a light source with better resemblance to the solar UVR spectrum.

Independent assessment of DNA damage from high exposures to Far-UVC in a human volunteer trial

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Keywords: ultraviolet; far-uvc; DNA damage; skin; (up to 4 words)

Background
Far-UVC (200-235nm) is a promising modality for indoor disinfection of airborne pathogens, causing DNA and protein damage to pathogens rendering them inert, while purportedly remaining safe for human skin and eye exposure [1]. A recent study (PIVUC) exposed healthy volunteers to high doses of Far-UVC to investigate possible upper limits of safe skin exposure. This study had two stages - in Stage 1 twelve volunteers were irradiated with 3000 and 6000 mJ cm-2 of Far-UVC and a 2x NB-UVB MED positive control, in Stage 2 eight volunteers were irradiated with a chronic 12000 mJ cm-2 exposure (1500 mJ cm-2 twice daily for four days) and a 2x NB-UVB MED positive control. No visible effects (e.g. erythema) after Far-UVC exposure were noted in the PIVUC study.

Objectives
To determine DNA damage via assessment of γH2AX in skin biopsies from the PIVUC study.

Methods
Skin sections were blinded during processing until all samples were analysed. Samples were stained with γH2AX antibody and DAPI, then analysed via mean fluorescence in ImageJ. Stratum corneum thickness was also measured.

Results
There are significant levels of γH2AX present in all exposure groups compared to unexposed. There is a (non-significant) difference between 3000 and 6000 mJ cm-2 groups indicating potential dose response. While there was significant difference between the single 3000 mJ cm-2 and NB-UVB exposures, there was no difference between the chronic 12000 mJ cm-2 and NB-UVB exposures, indicating an accumulation of DNA damage in repeat exposures. There was an association between increasing stratum corneum thickness and decreasing γH2AX for all Far-UVC exposures, but not for NB-UVB exposures.

Conclusions
Whereas previous assessment of DNA damage via detection of CPDs showed minimal damage only in the uppermost layers of the skin [2], in this study γH2AX is detected throughout the epidermis including the basal layer. Though these exposures far exceed the current ICNIRP and the ACGIH skin exposure limits (23 mJ cm-2 and 479 mJ cm-2 respectively, 222 nm unweighted), it is important to determine a threshold limit wherein Far-UVC exposure may be expected to cause harm. As such, work following on from this study will aim to replicate these responses in ex vivo tissue, and characterise a dose response curve for DNA damage caused by Far-UVC exposure.

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Light in dentistry
Chair: Martha Ribeiro

Advancing Dental Care with Nonthermal Light-Based Technologies

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Keywords: fluorescence, photodynamic therapy, photobiomodulation therapy

The field of dental care is undergoing a paradigm shift with the integration of nonthermal light-based technologies, offering innovative and noninvasive solutions for diagnosis, treatment, and prevention. This talk will explore key modalities such as photodynamic therapy (PDT), photobiomodulation therapy (PBMt), and optical diagnostics such as fluorescence imaging.

These technologies employ proper wavelengths of light to elicit targeted biological responses without generating heat, enabling safer and more precise interventions. PDT provides an effective antimicrobial approach for managing periodontitis, endodontic infections, and contaminated mucosal lesions. PBMt promotes tissue regeneration, reduces inflammation, and alleviates pain across a range of oral conditions. Fluorescence imaging enhances diagnostic accuracy by enabling real-time detection of biofilm and early oral cancer. Incorporating these nonthermal technologies into routine dental practice not only improves patient outcomes but also supports the shift toward personalized and preventive care.

This presentation will highlight clinical findings and emerging applications, emphasizing the transformative potential of nonthermal photonic technologies to redefine therapeutic protocols and advance long-term oral health.

Funding: FAPESP, CNEN and CNPq

Antimicrobial Photodynamic Therapy in the Oral Cavity: Impacts on the Oral Microbiome

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Keywords: oral microbiome; antimicrobial photodynamic therapy; dysbiosis; targeted aPDT.

The oral microbiome plays a crucial role in both oral and systemic health. This complex community of microorganisms contributes to immune homeostasis, metabolic regulation, and colonization resistance. Oral microbiome dysbiosis has been implicated not only in local pathologies such as caries, periodontitis, and peri-implantitis, but also in systemic diseases including cardiovascular disease, diabetes, adverse pregnancy outcomes, and neurodegenerative disorders. As a result, therapeutic strategies that promote a balanced oral microbiome are of increasing interest.

Antimicrobial photodynamic therapy (aPDT) is a promising adjunctive treatment for oral infections. It utilizes a photosensitizer, light, and oxygen to generate reactive oxygen species (ROS) that can inactivate microorganisms, with the important advantage of not inducing antimicrobial resistance. However, the susceptibility of oral microorganisms to photodynamic inactivation is species-dependent and influenced by biofilm architecture and ecological interactions.

Emerging research on “targeted aPDT” explores strategies to enhance the specificity of photodynamic action toward pathogenic species within dysbiotic oral biofilms. Approaches such as the conjugation of photosensitizers to species-specific binding domains (e.g., phage-derived cell wall binding domains) may enable selective targeting of pathogens like Porphyromonas gingivalis or Fusobacterium nucleatum, while minimizing disruption of beneficial commensal communities. Although this concept remains experimental, it offers a promising avenue for future precision modulation of the oral microbiome.

Future research is needed to fully understand the long-term ecological impacts of aPDT on the oral microbiome and to refine these targeted strategies for clinical use.

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Light in dentistry
Chair: Martha Ribeiro

The use of aPDT mediated by Butyl Toluidine blue to inactivate Candida albicans

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Keywords: Candidiasis, photosensitizer, fungal infection,

Candida albicans is a fungal organism that can shift from being a harmless commensal to a pathogenic agent, particularly in immunocompromised individuals. It frequently forms biofilms that increase its resistance to antifungal treatments. This study evaluates the antimicrobial efficacy of a novel photosensitizer, Butyl Toluidine Blue O (BuTBO), for the photodynamic inactivation of *C. albicans*. BuTBO, a phenothiazine-based photosensitizer, was tested for its ability to reduce fungal viability under various light irradiation conditions.

Methods: *Candida albicans* (ATCC 90028) was cultured on Sabouraud Dextrose medium and suspended in PBS to prepare an inoculum of 10^6 CFU/mL. BuTBO was dissolved in PBS to a final concentration of 100 µM and incubated with the yeast for 1 minute prior to irradiation with a 660 nm diode laser. Samples were collected after 1, 2, 3, and 5 minutes of irradiation for analysis.

Results: BuTBO-mediated antimicrobial photodynamic therapy (aPDT) significantly reduced yeast viability following laser irradiation. BuTBO uptake was confirmed through spectroscopy and fluorescence microscopy. However, prolonged incubation times did not improve efficacy, despite increased photosensitizer uptake.

Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test.
Conclusion: BuTBO-mediated aPDT effectively reduced *C. albicans* viability in a time-dependent manner. Its preferential accumulation in the cytoplasm highlights its potential as a therapeutic agent against resistant fungal infections.

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Light in dentistry
Chair: Martha Ribeiro

Proof-of-concept for systemic photodynamic therapy with methylene blue in a murine model of disseminated *Candida auris*

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Keywords: antimicrobial photoinactivation; critical priority pathogen; yeast infection; whole-body irradiation

The global emergence of *Candida auris* as a multidrug-resistant fungal pathogen has posed a significant challenge to current antifungal therapies, particularly in the context of systemic infections affecting immunocompromised patients. The ability of *C. auris* to colonize the bloodstream and internal organs, coupled with its high resistance to first-line antifungal agents, underscores the urgent need for alternative treatment strategies [1, 2]. Photodynamic therapy (PDT), which involves the use of light to activate a photosensitizer like methylene blue (MB) generating reactive oxygen species that kills microbial cells, has been widely explored for superficial and localized infections, but its systemic use remains limited due to challenges in light delivery and photosensitizer biodistribution [3, 4].

In this study, we assessed the proof-of-concept for systemic PDT by evaluating its efficacy in a murine model of disseminated *C. auris* infection (CBS 10913 strain). Female BALB/c nude mice were assigned to three groups (n = 4/group): (1) Control, (2) Free-MB and (3) MB-stained. In the MB-stained group, equal volumes of a fungal suspension (2×10^8 CFU/mL) and MB solution (1 mg/kg) were mixed and incubated for 20 min, resulting in a final suspension of 1×10^8 CFU/mL and MB at 0.5 mg/mL. A total of 200 µL of the mixture was intravenously injected via tail vein. In the Free-MB group, mice were first injected with 100 µL of the yeast suspension (2×10^8 CFU/mL), followed 20 min later by 100 µL MB at 1 mg/mL. Control mice received the same fungal inoculum, followed 20 min later by 100 µL of sterile phosphate-buffered saline (PBS). Forty minutes after the final injection, all animals underwent whole-body irradiation for 30 min using the LEDbox system (Biolambda, Brazil), delivering red light at 660 nm (50 mW/cm², 90 J/cm²). Blood samples were collected at baseline, 1 h and 24 h post-treatment. Fungal burden was quantified by serial dilution and plating. As shown in **Figure 1**, no significant differences were observed among groups at the 1 h timepoint. However, at 24 h post-PDT, both Free-MB and MB-stained groups showed significantly reduced fungal loads when compared to the Control group at 1 h. Although yeast counts at 24 h were similar in both MB-treated groups, the MB-stained cohort showed a significant baseline reduction, implying that intracellular MB accumulation potentiates PDT against systemic infections. Current studies are probing higher MB concentrations and membrane-permeabilizing adjuvants to further enhance uptake and refine the PDT regimen.

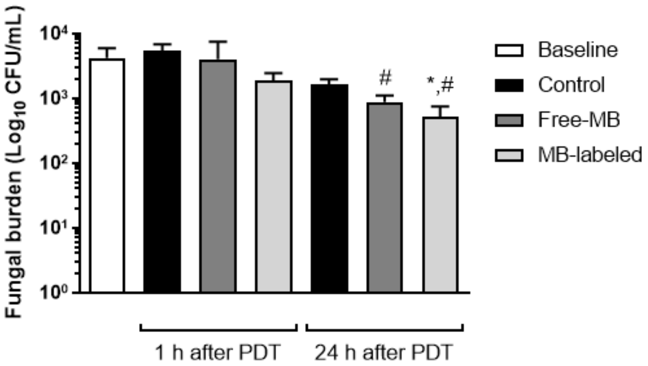

Figure 1. Fungal burden in peripheral blood of mice at baseline, 1 and 24 h after PDT. A significant reduction in fungal load was observed in the MB-stained group at 24 h compared to baseline (*, $p<0.05$), and in both Free-MB and MB-stained groups compared to the Control at 1 h post-PDT (#, $p<0.05$). Bars represent mean ± SD; one-way ANOVA followed by Tukey's post hoc test.

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The experimental protocol was approved by the Ethic Committee on Animal Use of the Nuclear and Energy Research Institute (CEUA-IPEN) and followed international guidelines for animal experimentation.

This work was supported by the National Council for Scientific and Technological Development (CNPq grant #420645/2023-3). ARS acknowledges support from the São Paulo Research Foundation (FAPESP, scholarship #2021/14119-6) and TMY from the National Nuclear Energy Commission (CNEN).

Photosensitized oxidation of free and peptide tryptophan to *N*-formylkynurenine

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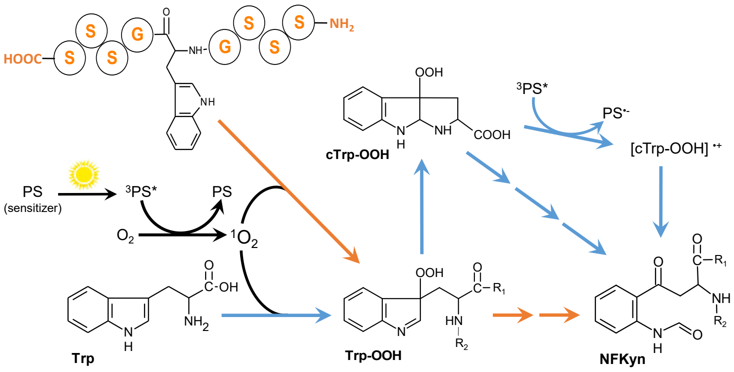
Keywords: Photosensitization; Proteins; Tryptophan; N-formylkynurenine

The oxidation of proteins and, in particular, of tryptophan (Trp) residues leads to chemical modifications that can affect the structure and function. The oxidative damage to proteins in photochemical processes is relevant in skin and eyes and is related to a series of pathologies triggered by the exposure to electromagnetic radiation. UV-A (320–400 nm) and visible radiation induce oxidation reactions through photosensitized processes.[1] Photosensitization of Trp is also important because in its degradation generates photosensitizers. This is a case in which products of a photosensitized process can act as photosensitizers themselves, leading to further photosensitized damage. In this context, *N*-formylkynurenine (NFKyn), product of the photosensitized oxidation of Trp, is an efficient photosensitizer able to be excited with UVA radiation.[2] In the oxidation of Trp by singlet oxygen ($^1O_2$) it has been proposed the initial formation of an unstable 3-hydroperoxytryptophan (Trp-OOH) *via* ene reaction, which then evolves to NFKyn in a complex series of competitive pathways.[3]

In this work we studied the photosensitized formation of NFKyn from Trp in different reaction systems. We used two substrates: free Trp and a peptide of nine amino acid residues, Trp being the only oxidizable residue. Two different photosensitizers were employed: Rose Bengal (RB) and pterin (Ptr). The former is a typical type II photosensitizer (acts by producing $^1O_2$), whereas Ptr is the parent compound of oxidized or aromatic pterins, natural photosensitizers that accumulate in human skin under certain pathological conditions and photosensitizes the oxidation of Trp mainly through type I mechanisms (generation of radicals).[4] Experimental data were collected in steady photolysis and the irradiated solutions were analyzed by chromatography (HPLC). Results indicate that the reaction of Trp with $^1O_2$ initiates the process leading to NFKyn, but different competitive pathways take place, depending on the photosensitizer and the substrate (Scheme).[5] In Ptr-photosensitization, type I mechanism is involved in secondary reactions accelerating the formation of NFKyn when free Trp is the substrate.



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Light-Activatable Hyaluronic Acid-Derivatives Releasing Nitric Oxide and Their Delivery in the Skin

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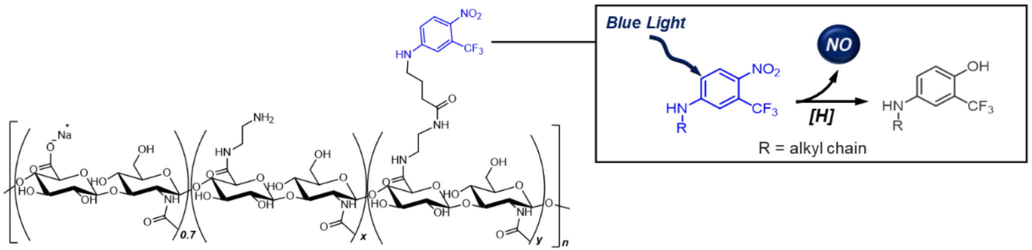
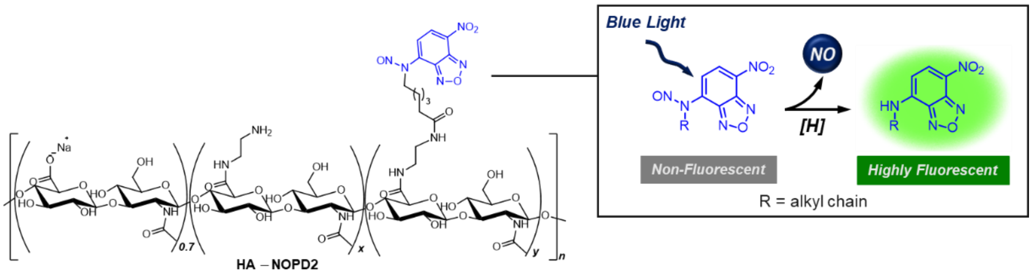
Keywords: hyaluronic acid, nitric oxide, light, microemulsions.

Hyaluronic acid (HA), widely known for its hydrating capabilities, can penetrate deeper into the skin in its low molecular weight form, promoting tissue repair and reducing inflammation [1]. Nitric oxide (NO), a gaseous signaling molecule involved in various physiological and pathological processes, is expected to enhance HA's effects in the skin through vasodilation, anti-inflammatory, and regenerative actions [2]. Here, we present a new class of HA-based compounds functionalized with NO photodonors (NOPDs) for light-triggered therapeutic use [3]. Upon blue light exposure, the synthesized HA derivatives HA-NOPD1 and HA-NOPD2 effectively released NO. In vitro assays on HaCaT keratinocytes showed enhanced cell proliferation and migration under light stimulation, underscoring the therapeutic potential of HA-NOPD1. The derivatives were formulated into microemulsions (MEs) to enable skin delivery. These photoresponsive MEs achieved efficient NO release under irradiation, with higher quantum yields than the unformulated compounds. Skin permeation studies on porcine and artificial membranes confirmed that HA-NOPD2 reached all skin layers, including the dermis, and released NO in situ.



HA – NOPD1



HA – NOPD2

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The PUVA (Psoralen + UVA) Process in the Light of Time-Resolved Spectroscopy

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Keywords: PUVA; DNA; time-resolved spectroscopy; photoinduced electron transfer; photo-addition

Furocoumarins – in particular psoralens – are herbal substances with a long pharmaceutical tradition (for structure see Figure 1) [1]. In the PUVA treatment of skin diseases, psoralen derivatives are administered and the affected skin region is exposed to UVA radiation [2]. Psoralens are known to target DNA by intercalation ensued by photo-addition to the DNA base thymine (see Figure 1) [3]. Angelicins are angular furocoumarins that also interact with DNA, yet have not found broad pharmaceutical applications as psoralens [4]. Here, we summarize our efforts to scrutinize the underlying molecular mechanisms of furocoumarin-DNA interactions using time-resolved spectroscopy and outline future directions.

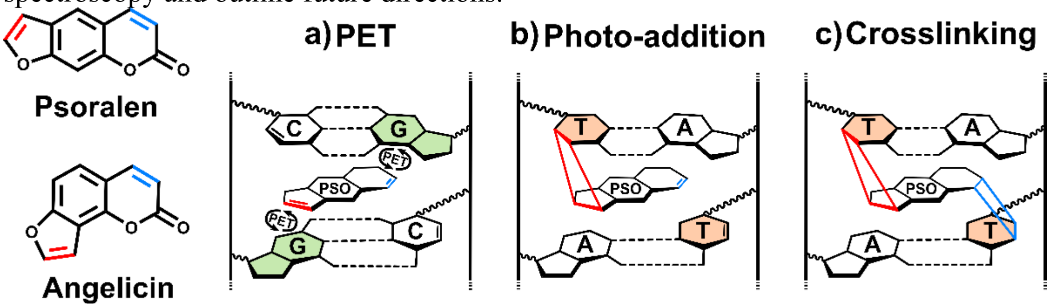

Figure 1. Structures of relevant furocoumarin parent compounds and photo-induced processes investigated. (a) Photo-induced electron transfer between guanine and excited psoralen. (b) Photo-addition of a psoralen to the DNA base thymine. (c) Crosslinking of DNA by psoralen.

Experiments on psoralens intercalated into guanine-containing DNA revealed a photo-induced electron transfer (PET) competing with the photo-addition (Figure 1a) [5,6]. The actual photo-addition to thymine was shown to proceed *via* the triplet state of psoralen in a stepwise fashion (Figure 1b) [7]. Preliminary experiments on the secondary photochemistry of psoralens in DNA, namely the crosslinking (Figure 1c), suggest that this process also involves the triplet state. With the first experimental results on angelicins interacting with DNA the talk will end.

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Thermodynamics of the Amotosalen-DNA Intercalation

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Keywords: Psoralens; Intercalation; Isothermal Titration Calorimetry; **Molecular Dynamics Simulations**

Autoimmune skin disorders are commonly treated with PUVA-therapy (psoralen + UVA-light) [1]. The synthetic psoralen derivative amotosalen (AMO) is used in the inactivation process of pathogens in blood products, capitalising on its strong photosensitizing effect after excitation [2]. The mode of action consists of two successive steps: AMO enters the pathogen's cell and reversibly intercalates into the base pairs of the helical regions of the DNA strands. Then, photoexcited AMO covalently binds to thymine (T) bases, *via* a biradical [3]. The resulting monoadduct (and interstrand cross-links, if a second photon is applied) leads to photoinduced damages in the pathogen's DNA and subsequent apoptosis [1].

Psoralens, usually "suffer" from a competing reaction with adjacent guanine (G) bases. This energetically favourable photoinduced electron transfer reaction (PET) reduces the cytotoxic effect of psoralens [4]. Several approaches to reduce the PET impact during the psoralen-DNA interaction have been explored in the past [5], mainly focusing on the PET energetics.

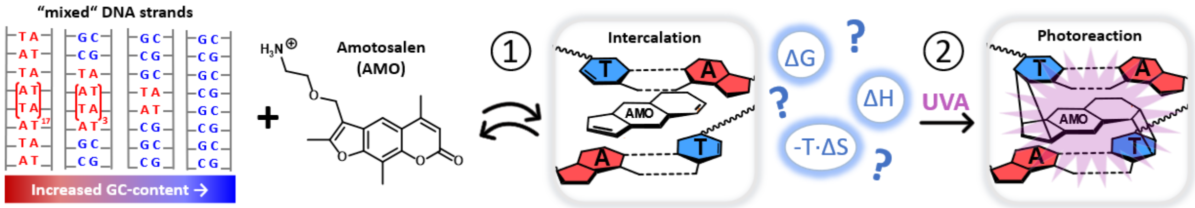

Figure 4: Interaction of amotosalen (AMO) and different "mixed" DNA oligomers. ① Reversible intercalation reaction. ② UVA-induced covalently linked interstrand cross-links with thymine bases.

Here, the recently found unique capacity of AMO to intercalate preferentially into long AT-DNA strands, compared to short GC-strands [6], is explored by means of Isothermal Titration Calorimetry (ITC) **in combination with Molecular Dynamics Simulations and Molecular Docking.** A favourable free binding energy in AT-intercalation sites might lead to a higher cytotoxicity of new psoralen structures, evading the PET process [7]. Thus, quantification and optimization of the thermodynamics of intercalation might yield more efficient PUVA agents.

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Photosensitization by topical retinoids: 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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Chemico-physical and biological properties of two monoclonal antibodies, Bevacizumab (Avastin®) and Durvalumab (Imfinzi®) under real-life light doses.

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Keywords: Bevacizumab; Durvalumab; Photostability; Real-life

INTRODUCTION. Monoclonal antibodies (mAbs) have recently gained prominence as protein drugs in the treatment of different kinds of cancer. Because of their protein nature, they can be unstable to some stressors during manufacturing, transportation, storage, handling/dilution, and administration [1]. Light can induce chemico-physical changes which can be relevant when amino acids are in the CDRs, as efficacy and safety could be compromised [2,3]. **AIMS.** Chemico-physical stability of Bevacizumab-Avastin® and Durvalumab- Imfinzi®, and their biological activity were evaluated under light doses close to the exposure of mAbs in their real-life. **RESULTS.** Exposure of the diluted mAbs to a dose of sunlight experienced in their real life did not affect their conformation. On the other hand, light was able to induce mAb aggregation, at a higher extent in Bevacizumab than in Durvalumab. The chemical modifications detected on both mAbs revealed low oxidative damage plus deamidations. Chemico-physical modifications on both Bevacizumab and Durvalumab had little effect on their target recognition (VEGF and PDL1, respectively). Furthermore, immunogenic potential in dendritic cells from differentiated monocytes seems absent. **CONCLUSIONS.** The chemico-physical changes induced by real-life doses of light on the tested mAbs are not crucial for the overall protein structure and very low chemical modifications involve amino acids located in the CDRs. Consequently, in vitro target recognition barely decreases. Moreover, the detected aggregation does not seem to induce immunogenicity but to have a role on the decrease of the biological activity.

AKNOWLEDGEMENTS This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement N° 101007939 (RealHOPE).

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Uncovering Mutagenic Cascades: A UV-Triggered Crosslink Between Etheno-Cytosine and Thymine Drives Additional DNA Lesions

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Keywords: Cyclobutane pyrimidine dimer, etheno adduct, mutagenic properties, DNA lesions

Lipid peroxidation is a biochemical process that involves the oxidation of polyunsaturated fatty acids, the components of cell membranes. In this process, highly reactive species, such as α,β-unsaturated aldehydes, are generated and react with DNA bases, forming the so-called **etheno adducts**. These DNA lesions exhibit **mutagenic properties** [1]. Indeed, the increase of lipid peroxidation in inflammatory processes[2] has been related to the high levels of etheno adducts in diseases that can lead to cancer, such as Chron's disease or ulcerative colitis[1]. Consequently, they are excellent **bio-markers** for different types of cancer (lung, colon or prostate cancer). Although their mutagenic properties have been clearly established, their photoreactivity has not been studied yet.

Here, the attention is centered on the cytosine etheno derivative (eC). Recently, the increase of its singlet excited state lifetime compared to that of cytosine was reported,[3] which points toward a **potentially higher reactivity**. Two model compounds containing two eC or eC and thymine covalently linked by a trimethylene bridge were synthesized and their photoreactivity was evaluated under **direct irradiation** or through a **photosensitization** process. Interestingly, under both conditions, a sole photoproduct, characterized as a cyclobutane pyrimidine dimer-like compound, was evidenced for the first time. As in the case of its cyclobutane pyrimidine dimer (CPD) analogue, formation of this lesion might arise from a **[2+2] photocycloaddition** involving the double bond at 5,6 of the thymine and eC. In addition, mechanistic insights were obtained from emission and transient absorption experiments.

Formation of edC already means a damage by itself, but its harmfulness could be increased if it can form a CPD-type photoproduct. Thus, the aim of this study is to evaluate the photoreactivity of these etheno adducts, considering especially the formation of new CPD-like lesions.

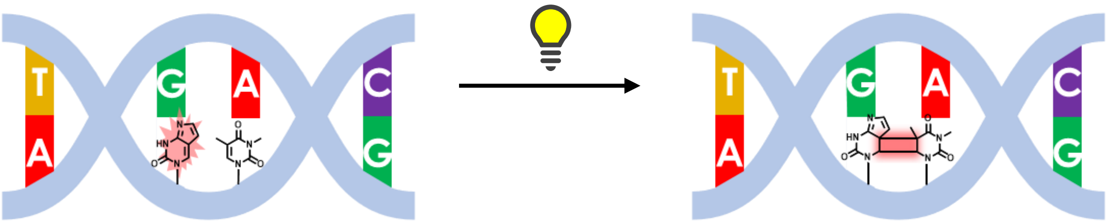

Figure 1:
Graphical representation of the eC-T model and its CPD formation

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"From Reduced to Oxidized: How Eumelanin Oxidation State Modulates Excited States and Photoprotective Efficacy"

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Keywords: melanin; carotenoids; singlet oxygen, excited states

Human retina is at elevated risk of oxidative stress due to exposure to light (above 3 eV) from focused irradiation and high oxygen tension [1]. Carotenoids such as lutein and zeaxanthin, and melanin are involed in photoprotection of the retina [2]. In human postmitotic cells, such as retinal pigment epithelium (RPE) cells, melanin is synthesized early during fetal development and gradually degrades with age. Age-related oxidative modifications of RPE melanin are thought to be the result of photochemical processes. Importantly, oxidatively degraded melanin shows increased photochemical activity, including increased efficiency of singlet oxygen photogeneration [3]. This suggests that melanin subunits in different oxidation states exhibit different energy levels for their first excited states, in particular the first singlet excited state (S_1) and the first triplet excited state (T_1).

In this study, we aimed at determining the first excited state energies for eumelanin models in different oxidation states: reduced form hydroquinon (H_2Q), partially oxidized form quinone (IQ) and fully oxidized form quinone imine (QI). Monomeric models, dimeric structures and stacked dimer systems were investigated. Adiabatic transitions were calculated using the CAM-B3LYP function, with water modeled as a solvent in the solvation continuum model, and also using the def2-TZVP basis set. Furthermore, for comparative purposes, excited state energies were determined for retinal carotenoids (lutein and zeaxanthin) using tetrahydrofuran as solvent.

Our results confirmed that oxidized melanin subunits exhibit lower first excited state energies compared to their reduced counterparts. Interestingly, the excited state energies for the oxidized forms were comparable or even lower than those for lutein and zeaxanthin — carotenoids known for their effective ability to quench singlet oxygen. This is due, among other things, to their low-energy triplet states (T1) capable of quenching singlet oxygen (approx. 1 eV). This finding suggests that oxidized forms of eumelanin can actively participate in quenching of singlet oxygen.

Additionally, we found that DHI-based models were more sensitive to changes in the excited state energy associated with oxidation than DHICA-based models.The obtained results suggest that one of the mechanisms of eumelanin photoprotection may be related to the quenching of singlet oxygen via triplet-triplet interactions interactions as presented in [4] [5].

Future studies can be extended to search for melanin modifications that may be associated with a change in the pigment properties towards pro-oxidant properties

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Carnosine oxidation mediated by singlet molecular oxygen

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Keywords: singlet oxygen; carnosine, mass spectrometry

Endogenous histidine-containing dipeptides, such as carnosine (β-alanyl-L-histidine, CAR), homocarnosine (γ-amino-butyryl-histidine), and anserine (β-alanyl-L-1-methylhistidine), have been recognized as detoxifying agents against reactive oxygen species. Carnosine is present in high concentrations in skeletal muscle and in the central nervous system. We have recently observed a decrease in carnosine levels in the muscles of rats with aging. A statistically significant decrease was also observed in the symptomatic 120-day-old ALS (amyotrophic lateral sclerosis) rat muscle compared to controls of the same age [1]. Interestingly, a decrease in carnosine tissue concentrations was also observed in muscle biopsies of ALS patients. Recently, singlet oxygen-mediated oxygenation and the subsequent carnosine reaction were analyzed at the molecular level. It was found that a cyclic homodimer is one of the end products after the photooxygenation of carnosine. These authors observed the same transformation in pork specimens [2]. In this work, we investigated the reaction products of singlet molecular oxygen with carnosine using on-line reverse-phase high-performance liquid chromatography with electrospray ion-trap mass spectrometry detection. Carnosine (20 mM) was dissolved in deuterium oxide containing 10 μM methylene blue and irradiated using light from a tungsten lamp (500 watts) filtered through a 360 nm cutoff filter. The reaction products were isolated by reverse-phase HPLC and characterized by ESI/MS spectral analysis. The photosensitization of carnosine produces oxygenated products analogous to those proposed for free His residues. Understanding these mechanisms may contribute to clarifying the antioxidant role of carnosine and support the development of new therapeutic strategies.

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A Kinetic Approach to the Photoinduced Crosslinking of Unmodified Bovine Serum Albumin: Functional Consequences

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Keywords: PICUP, Photocrosslinking, Photooxidation.

The photoinduced crosslinking of unmodified proteins (PICUP) [1] is a powerful tool to generate protein-based nanostructures without prior chemical modification. In this work, we applied the PICUP reaction to bovine serum albumin (BSA) in air-saturated phosphate buffer, using ruthenium(II) tris(bipyridine) ($Ru(bpy)_3^{2+}$) as a photosensitizer and persulfate anion ($S_2O_8^{2-}$) as an electron acceptor. Upon blue light excitation, redox-generated radicals triggered covalent BSA oligomerization. Under aerobic conditions, however, singlet oxygen ($^1O_2$) is also produced, leading to the oxidation of electron-rich amino acid side chains [2]. The formation of dityrosine crosslinks and Trp oxygenation products such as N-formylkynurenine was investigated by steady-state and time-resolved fluorescence spectroscopy and UV-Vis absorption, which revealed characteristic signatures of these modifications. Our results show that aerobic PICUP yields BSA nanoparticles of ~100 nm and induces both oxygen-independent crosslinking and oxygen-mediated oxidative modifications.

Functionally, we observed a significant decrease in the esterase-like activity of the modified protein species: oxidized monomers retained 49% activity, and oligomers only 27%, compared to native BSA. These findings suggest that $^1O_2$-mediated oxidation and crosslinking act synergistically to impair protein function, likely due to structural alterations near the active site and steric hindrance caused by oligomerization. On the other hand, these crosslinked BSA nanostructures proved capable of encapsulating hydrophilic photosensitizers such as rose bengal, highlighting their potential as protein-based carriers in light-driven applications.

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Photoactivation and quenching mechanism of Orange Carotenoid Protein from multiscale simulations

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Orange carotenoid protein (OCP) is a photoreceptor responsible for photoprotection in cyanobacteria by quenching the excitation energy in the phycobilisome (PBS) antenna megacomplex. Strong blue-green light illumination by the bound keto-carotenoid triggers the activation of OCP, which is known to proceed through carotenoid translocation and results in the separation of N-terminal (NTD) and C-terminal domains (CTD) of OCP.[1,2] However, the details of both OCP activation and its function as a quencher of PBS fluorescence are still not clear. Specifically, the low yield of the primary photoproduct hinders a complete spectroscopic investigation of the very first steps of OCP photoactivation.[3]

Here I will show how multiscale calculations — combining enhanced sampling molecular dynamics, quantum mechanics/molecular mechanics (QM/MM) calculations, and nonadiabatic excited-state simulations — can help shed light into OCP activation and mechanism of photoprotection [4-7]. Our simulations elucidate the nature of the carotenoid's excited states in the photochemistry of OCP, and suggest a photoisomerization mechanism as the photochemical trigger promoting its activation [4,5]. Furthermore, we show that the carotenoid must translocate into the NTD to allow domain separation [6]. Finally, we show that excitation energy transfer from the phycobilin pigments to the S_1 state of the carotenoid is the likely quenching mechanism [7].

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Quantum-classical dynamics in the study of scotopic and photopic vision

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Keywords: Vision; Rhodopsin; Retinal Chromophore; Quantum Classical Trajectories

In this contribution we will show how, by using hundreds of quantum-classical trajectories combined with multiscale geometry optimization, it is possible to study the primary event in vision at both the molecular and electronic levels. [1-3] The lecture will present simulations for visual photoreceptor proteins responsible for scotopic and photopic vision in both vertebrate (a cow) and invertebrates (a squid and a spider). [4] We will also see how it is possible to use the same technique to get information on the vision of an extinct vertebrate (Archosaurus).

Content: We use multiscale modeling based on multistate multiconfigurational quantum chemistry to investigate natural and synthetic biological photoreceptors.

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Molecular modelling of the last steps of the Kok-Joliot cycle in Photosystem II

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Keywords: Oxygen Evolving Complex, Reaction Mechanisms; Molecular Dynamics; Quantum Chemistry

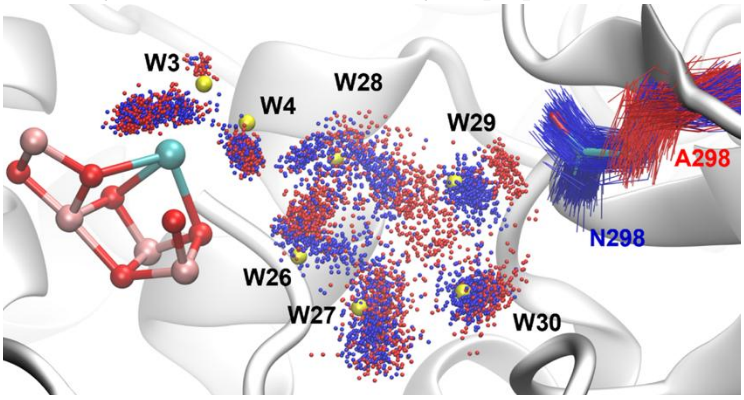
EXTENDED ABSTRACT

The catalytic cycle of the oxygen evolving complex (OEC) placed in the reaction centre of the Photosystem II (PSII) enzyme, leading to the water splitting into protons, electrons and molecular oxygen, consists of five consecutive steps, namely S0 – S4.

Recently, a study based on the combination of IR spectroscopy experiments and DFT-based calculations was able to identify the slowest step in photosynthetic O2-formation, after the S3 state and largely due to an entropic slowdown. [1] Nevertheless, the origin of such an entropic slowdown at molecular level is still largely unknown. While the OEC of the PSII is normally simulated at the quantum mechanics (QM) level, classical molecular dynamics (MD) simulations can provide the missing information about the specific positioning of water molecules and hydrogen bonding interactions around the Mn4Ca cluster in PSII on timescales inaccessible to QM calculations.

In this presentation, based on a multilevel theoretical approach, we will first show the series of events occurring during the S3 to S0 transition, focusing on the structural and energetic properties of the different intermediate steps [1-2]

Thereafter, by means of classical all-atoms MD simulations on the µs timescale, we will give hints on the origin of the entropic contribution associated with the bottleneck of photosynthetic oxygen evolution. The effect of mutations is also investigated [3].



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Beyond a single excitation: calculating multi-exciton dynamics and interaction in highly nonlinear spectroscopy of molecular photosystems

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Keywords: Ultrafast spectroscopy, Excitation transport, Photosynthesis

Recent advances in time-resolved nonlinear spectroscopy have shifted the attention to multi-excitonic dynamics and interaction, especially exciton–exciton annihilation[1-3]. Apart from being of interest by itself, the exciton–exciton annihilation allows tracking of the excitation transport over mesoscopic distances in on-average energetically flat landscape [1,4,5].
To interpret the highly nonlinear spectroscopy signals, a theoretical description of multiple interacting excitons is needed. In small systems, the states can be considered explicitly at the microscopic level within a coherent dynamics picture, with the nonlinear signal calculated by nonlinear response functions[4]. In contrast, in large systems a proven description is that of excitation density and diffusive transport, with high-order signal evaluated in terms of annihilated excitons without spectral resolution [3,5].
Bridging these two levels of description remains an open question. Is it possible to derive the quantities of the continuum description from microscopic quantum state structure and dynamics[4]? What are the effects of exciton delocalization[2]? Can highly nonlinear transient absorption follow interacting excitons in space and time? How detailed description is needed to extract quantities such as diffusion coefficient? Connecting the continuum diffusion model with Liouville-space pathway formulation, I will attempt to answer such questions.

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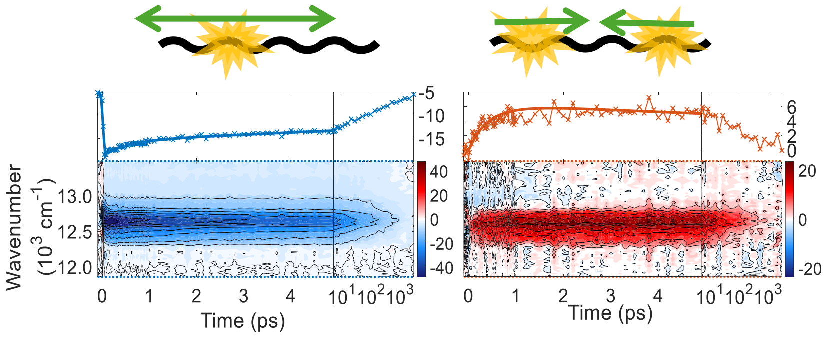

Modelling the interplay between structure and energy transfer in biosystems

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Figure Nonlinear spectroscopy data report on single-exciton dynamics and multi-exciton interaction. Only multi-exciton calculations can say what the data tell us about exciton energy, transport and interaction timescale.

Keywords: energy transfer, photosynthesis, disordered proteins, multiscale modelling

EXTENDED ABSTRACT

Modelling the interplay between electronic energy transfer and structure in biosystems is a considerable challenge due to the multiple space and time scales involved in the process.[1] Depending on the specific case under study, different protocols can be adopted often combining classical simulations with multiscale quantum/classical methods. In this contribution we will overview two challenging cases, the modelling of light harvesting and energy transfer in cryptophyte pigment-protein photosynthetic complexes, and the simulation of Förster resonance energy transfer (FRET) experiments on the partially disordered protein calmodulin.

First, we investigate how protein thermal fluctuations tune exciton states in the phycocyanin PC645 and PC612 antenna complexes of cryptophytes.[2] We report a dual molecular dynamics (MD) strategy that combines classical and quantum/classical Born-Oppenheimer simulations, and show that, whereas thermal effects have a small impact on the energy ladder in PC612, they are key to describe the properties of PC645, due to the absence of a hydrogen bond controlling the twist of ring D in PCB β82 bilins. Second, we investigate how structure determines the FRET properties of the partially disordered protein calmodulin, with a particular focus on the impact of Förster approximations regarding dielectric screening effects.[3] To characterize the conformational ensemble, we combine extended MD simulations in the microsecond timescale with a rigorous theoretical framework beyond Förster dipole approximation based on electrostatic potential-fitted transition charges coupled to an atomistic polarizable classical environment.[4] Our results indicate that screening effects depend on donor-acceptor separation and solvent exposure, significantly biasing the characterization of the disordered ensembles even at long donor-acceptor separations.

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First-principles simulations unveil the effect of mutations on light harvesting

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Keywords: light harvesting, mutation, simulation, spectroscopy

Natural light harvesting is exceptionally efficient thanks to the optimal arrangement of pigments within light-harvesting complexes (LHCs). To understand the design principles underlying energy transport in LHCs, ultrafast spectroscopy is often complemented with mutational studies that introduce perturbations to the excitonic structure of the natural complexes. However, the presence of many pigments and overlapping spectral bands makes it difficult to disentangle all spectral contributions and their changes upon mutation. Moreover, multiple excitation energy transfer (EET) processes occur on the same timescale, leading to intricate dynamics of the time-resolved spectral features. Simulations help to disentangle these signals, providing insights into the complex photophysics in LHCs. However, experimental spectra are often fitted to obtain the microscopic model used to simulate EET and spectroscopies in LHCs. Although inexpensive, this approach may lead to a biased microscopic model due to error compensation and lacks a direct link between the energy transfer network and the molecular structure.

Here, we demonstrate how a synergistic combination of first-principles simulations and transient absorption (TA) spectroscopy can provide unprecedented insight into EET pathways in LHCs. We focus on the Chlorophylls (Chls) network in CP29, a minor LHC of plants [1] and two of its mutants [2,3].

To validate our computational model, we simulate the TA spectra of the Chls network and compare with experimental measurements [4]. The good agreement between theory and experiment supports the accuracy and predictive power of our model. After validating the model, we systematically map each experimental kinetic component to a specific EET network [2]. Our model allows us to identify the Chl acting as bottleneck for the EET network in CP29, and those acting as terminal emitters for the excitation energy. We explain the changes observed upon mutation at molecular detail, thanks to the first-principles nature of our simulations. Our combined strategy reveals that EET in CP29 is surprisingly robust to the changes in the exciton states induced by mutations, highlighting the adaptability and versatility of plant LHCs.

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Molecular determinants of the mechanism of action of PSBS under the computational microscope

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Keywords: Photoprotection; Molecular Dynamics;

Plants require sunlight to survive. However, upon a sudden increase of light intensity, an excess of photoexcitations in the photosynthetic membrane (thylakoid) may cause damage via photooxidation. To avoid this, photosynthetic organisms activate a mechanism called energy dependent quenching (qE) to protect themselves [1].

The sites of this activation are Light Harvesting Complexes (LHCs), membrane protein complexes that bind chlorophylls and carotenoids and are located in the thylakoid membrane [2]. The activation of qE in the LHCs is triggered by the acidification of the thylakoid lumen. As the LHCs are not able to sense the acidification, they require external triggers [3]. In plants the trigger is the pigment-less protein PsbS [4] which is able to sense and respond to changes of pH via conformational changes [6]. However, what is the mechanism of interaction of PsbS with the LHCs? How does PsbS-LHCs interaction activate qE?

We here used a combination of state-of-the-art molecular dynamics methods to study the mechanism of action of PsbS on the LHCs across different levels of resolution. We find that instead a simple binary switch, PsbS acts with a more nuanced mechanism to fine tune its effects on the membrane and the LHCs.

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Computational Insights into the dynamic interaction of FCP-LHCX1 complexes in Diatoms.

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Keywords: Diatoms, photoprotection, FCP-LHCX1 interaction, molecular dynamics

Diatoms are major contributors to global oxygen production and carbon cycling, possess exceptional light-harvesting and robust photoprotective mechanisms [1]. Under high or fluctuating light conditions, they activate photoprotective mechanisms (non-photochemical quenching or NPQ) to dissipate excess excitation energy as heat within their light harvesting complexes Fucoxanthin and chlorophyll a/c binding proteins, or FCPs) [2]. The LHCX family of proteins is essential for this photoprotective mechanism, in synergy with factors like the xanthiphyll cycle and transthylakoid ΔpH [3]. Within this synergy, FCP complexes could associate with LHCX proteins, however solid structural and dynamic details of an FCP-LHCX1 interaction are not available. In this study we have employed a computational approach to investigate the dynamics of different FCP-LHCX1 complexes from three diatom species (*Phaeodacttylum triconotum, Cyclotella meneghiniana, Chaetoceros gracilis*) at different ΔpH states. We have run AlphaFold-based structure predictions and classical molecular dynamics (MD). The analysis is focused on how lumen acidification (from pH 7.0 to 5.5) LHCX1 binding to FCPs and the xanthophyll cycle, specifically the conversion of diadinoxanthin (Ddx) to diatoxanthin (Dtx), triggers conformational changes in FCPs that could potentially be correlated with photoprotection. Our results reveal pH-dependent conformational transitions indicative of a functional switch from light harvesting to energy dissipation, highlighting specific residues and pigments as key sites for quenching. We propose a structural model for the FCP-LHCX1 complex, highlighting conserved interactions and conformational signatures that align with previous findings demonstrating strong FCP-LHCX1 synergy in energy quenching [4]. By advancing the molecular understanding of NPQ in diatoms we offer potential targets for mutagenesis to engineer improved variants, with broader implications for global CO_2 assimilation and bioinspired photosynthetic systems.

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Acknowledgment: This project has received funding from Horizon Europe (HORIZON) within the scheme of Marie Skłodowska-Curie Actions Doctoral Networks (MSCA-DN) with grant agreement ID: 101119442 and title PhotoCaM - "Photosynthetic Antennas in a Computational Microscope: Training a new generation of computational scientist"

Prediction of non-additive effects of complex UV sources: application to DNA photochemistry

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Keywords: DNA photochemistry, data modeling, action spectra, experimental design

EXTENDED ABSTRACT

A growing number of experimental evidence emphasizes that photobiological phenomena are not always the sum of the effect of individual wavelengths present in the emission spectrum of light sources [1]. Consequently, the classical action spectrum approach may be misleading in some cases. Unfortunately, tools are missing to identify such non-additive effects and predict the effects of various exposure conditions. In the present work, we addressed these points for the formation of pyrimidine dimers in DNA upon co-exposure to UVC, UVB and UVA radiation.

DNA photochemistry is a valuable model for this topic since all possible “cocktail effects” are expected. Cyclobutane pyrimidine dimers (CPDs) are produced in the three wavelengths’ ranges in an additive manner. Pyrimidine (6-4) pyrimidone photoproducts (64PPs) are also produced in a dose-dependent way but they are readily converted into Dewars valence isomers (Dewars) upon absorption of UVB and UVA. Consequently, formation of 64PP undergoes an inhibitory effect in combination of UVC or UVB with UVB or UVA, while a synergistic effect is observed for Dewars.

The yields of these photoproducts were investigated with two mathematical tools. The first was the combination index, a method initially developed for the study of combinations of drugs. We showed that this approach could be used to determine whether mixtures of theses UV ranges exhibited additive, inhibitory or synergistic effects [2,3].

In a subsequent step, we developed a strategy based on experimental design, which is commonly used for the optimization of multi-parameters industrial processes. We could show that this approach is very efficient to quantify the contribution of each wavelength range, and of their interactions, to the formation of DNA photoproducts [3]. The obtained models allowed us to accurately predict the level of pyrimidine dimers in DNA irradiated under different conditions. The data were found to be more accurate than those obtained with the simple additive approach underlying the use of action spectra. Both combination index and experimental design thus appear as attractive concepts for the study of complex light-driven phenomena. They could be widely applied in photobiology even for cellular experiments.

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An N-Doped Carbon Dots Nanoconjugate for the Simultaneous Generation of Nitric Oxide and Singlet Oxygen with Green Light

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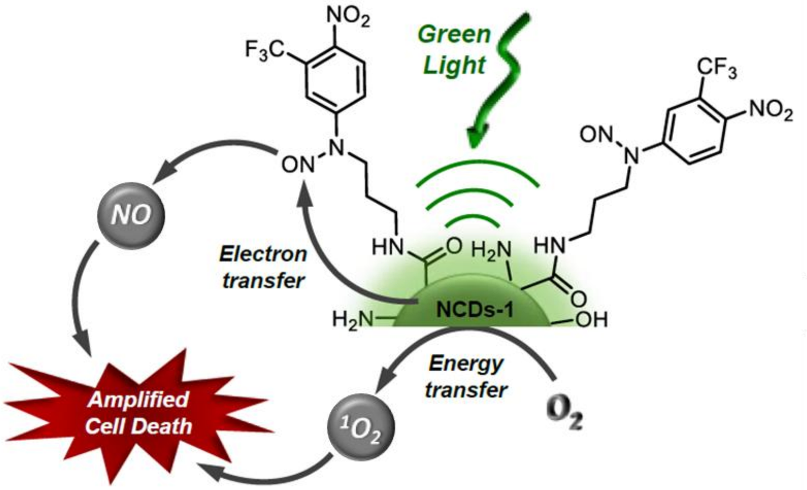
Keywords: Nitric Oxide; Singlet Oxygen; Carbon Dots; Photodynamic Therapy

Nitric oxide (NO) and singlet oxygen ($^1O_2$) are two highly promising agents for innovative phototherapeutic strategies in cancer treatment [1-3]. In this study, nitrogen-doped carbon dots (NCDs) with strong absorption in the biocompatible green region have been synthesized and covalently functionalized with an NO photodonor (NOPD) that typically requires blue light for activation, resulting in a nanoconjugate approximately 3.5 nm in size. The NCD core serves as a green light-harvesting antenna, enabling NO release from the NOPD through an intramolecular photoinduced electron transfer mechanism, effectively shifting the activation wavelength by over 100 nm. In parallel, green light excitation leads to the generation of $^1O_2$ via collisional energy transfer to molecular oxygen. Thanks to their intrinsic fluorescence, the nanoconjugates can be tracked within 9L/LacZ brain cancer cells, where they predominantly accumulate in the cytoplasm. Upon green light exposure, a significant increase in cancer cell death is observed, attributed to the synergistic photodynamic effects of NO and $^1O_2$.



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Assessment of cationic phthalocyanines and doxycycline against resistant microbes – innovative approach or dead end?

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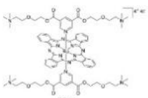
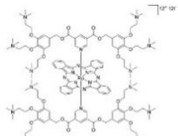
Keywords: phthalocyanines; doxycycline; MDR; PACT

Two sets of phthalocyanine-based compounds (PS1a–PS4a and 1–5), developed at the Autonomous University of Madrid, were evaluated for their photodynamic activity against multidrug-resistant pathogens. The Ru(II) complexes PS1a–PS4a, bearing multiple ammonium groups and displaying high singlet oxygen yields, showed strong activity against MRSA and *E. coli* under low light doses. Their ability to penetrate and bind to cell wall and membrane structures was confirmed, with amphiphilic derivatives (PS3a, PS4a) designed for enhanced intracellular targeting. Notably, PS4a exhibited synergism with subinhibitory doxycycline, increasing MRSA reduction from 1.5 to 4.9 logs. Compounds 1–5, functionalized with pyridyloxy and bulky hydrophobic substituents, demonstrated broad-spectrum efficacy against *S. aureus*, *MRSA*, *E. faecalis*, *E. coli* (including ESBL-producing strains), *P. aeruginosa* (including carbapenem-resistant), *C. albicans*, and *T. mentagrophytes*, achieving reductions of 1.4–5.6 logs. Several derivatives also showed enhanced antimicrobial effects in combination with doxycycline. These results highlight the therapeutic potential of cationic phthalocyanines in photodynamic control of resistant pathogens.


PS1a


PS2a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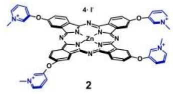
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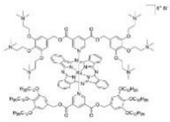
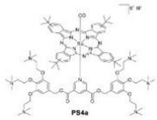
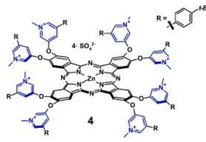

PS3a


PS4a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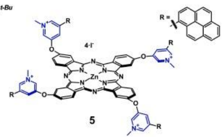
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Fluorescence lifetime imaging microscopy FLIM with BODIPY2 molecular rotor used to measure cancer cell membrane microviscosity

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Keywords: plasma membrane microviscosity, molecular rotors, fluorescence lifetime imaging microscopy FLIM

Introduction: An important biophysical parameter of living cell membranes is microviscosity, which contributes to membrane permeability, diffusion and transport rates, and the activity of membrane proteins. However, the relationship between the microviscosity of a cancer cell membrane and the response to therapy remains poorly understood. The study of microviscosity in living cell membranes has become possible due to advancements in bioimaging methods, one of the most promising being the application of fluorescence lifetime imaging microscopy FLIM with molecular rotors.

The aim is to study the role of membrane microviscosity in the response of cancer cells to chemo- and photodynamic therapy.

Materials and methods: The membrane microviscosity was analyzed in live cells and in vivo tumors using FLIM and the viscosity-sensitive fluorescent probe BODIPY2. For chemotherapy, Cisplatin and Oxaliplatin were used. For photodynamic therapy, the photosensitizer Photoditazine and genetically encoded photosensitizer KillerRed were employed. The study was conducted on cell cultures colorectal human cancer HCT116 and human cervical cancer HeLa Kyoto, 3D tumor spheroids, and mouse tumor models. To assess the lipid profile of membranes, TOF-Sims time-of-flight secondary ion mass spectrometry was used.

Results: It was found that membrane microviscosity increased upon treatment with platinum-based chemotherapeutic agents (Cisplatin and Oxaliplatin), which correlated with an increased number of dead cells [1]. The development of resistance to Oxaliplatin was accompanied by lipidome reorganization and restoration of microviscosity. Thus, changes in membrane microviscosity depend on the mechanism of action of the chemotherapeutic agent. During photodynamic therapy with the photosensitizer Photoditazin, a decrease in membrane fluidity was observed, associated with the initiation of lipid peroxidation [2]. When using the genetically encoded photosensitizer KillerRed, bidirectional changes in microviscosity were recorded depending on KillerRed's intracellular localization - either in the nucleus or in the plasma membrane [3].

Conclusion: The obtained data on membrane microviscosity correlated with low phosphatidylcholine signal and high cholesterol levels, along with a decreased amount of unsaturated fatty acids. The results demonstrate that maintaining constant membrane microviscosity through lipid profile remodeling plays a crucial role in drug resistance during carcinogenesis. Furthermore, the recorded changes in microviscosity upon therapeutic treatment may serve as a valuable tool for developing new and/or improving existing therapy protocols. This work was supported by the Russian Science Foundation (project No. 23-74-00045).

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Molecular and biological mechanism studies of protein photooxidation to control cell fate and their therapeutic applications

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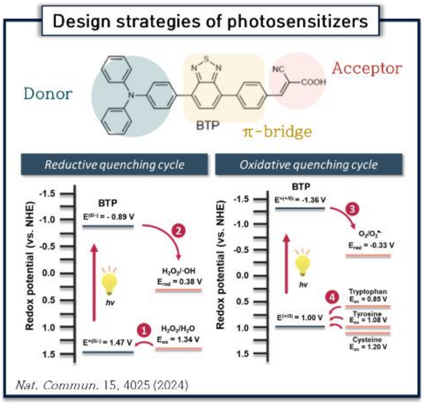
Keywords: Photodynamic therapy, Oxidation, Protein modification, Photosensitizer

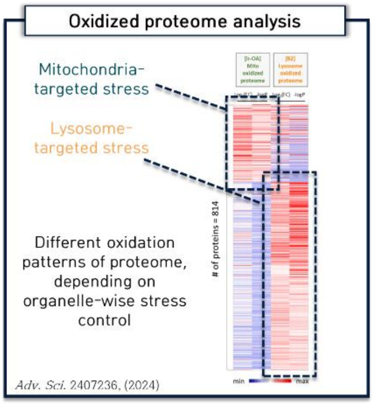
Reactive oxygen species (ROS) are increasingly recognized not only as damaging agents but also as modulators of cell fate, depending on their localization, dosage, and timing. This work explores how protein photooxidation, when spatially and chemically controlled, can be used to direct therapeutic outcomes in cancer and immune regulation.

We develop neutral Ir(III)-based photosensitizers that localize to lysosomes and generate ROS under light exposure.[1] This targeted lysosomal oxidation inhibits autophagy, a critical resistance mechanism in cancer cells. The approach sensitized drug-resistant cells in vitro and in vivo, offering a promising strategy to overcome therapeutic resistance via photoinduced autophagy disruption. We also introduces an amphiphilic photocatalyst that overcomes hypoxia—a major challenge in photodynamic therapy—by oxidizing intracellular water to H_2O_2, which forms hydroxyl radicals (•OH) via electron transfer.[2] The process is enhanced under hypoxic conditions and induces non-canonical pyroptosis by oxidizing membrane proteins, triggering inflammasome activation and gasdermin D-mediated cell death. This highlights the immunogenic potential of controlled protein oxidation.Lastly we uncovers a novel oxidation mechanism occurring within folded protein cores.[3] Through "O_2-confinement oxidation," molecular oxygen trapped in hydrophobic pockets reacts with photoexcited tryptophan to generate ROS, leading to structural collapse. Proteomics revealed this mechanism affects a broad set of cellular proteins, linking it to stress and degradation pathways.

These studies demonstrate that protein oxidation can be finely tuned to control subcellular processes and cell death pathways. Strategic design of photosensitizers enables therapeutic precision and opens new directions for treating resistant cancers and inducing immunogenic responses.

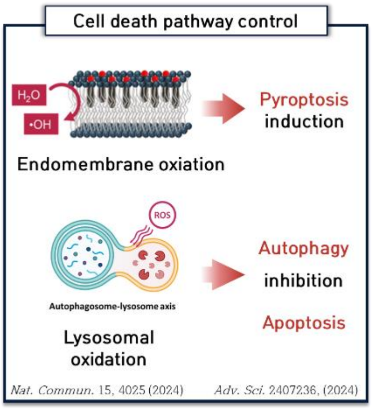

Design strategies of photosensitizers

Nat. Commun. 15, 4025 (2024)


Oxidized proteome analysis

Different oxidation patterns of proteome, depending on organelle-wise stress control

Adv. Sci. 2407236, (2024)


Cell death pathway control

Nat. Commun. 15, 4025 (2024) *Adv. Sci.* 2407236, (2024)

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Oral communications on PDT
Chair: Amparo Faustino

Novel Peptide-Based Prodrugs for Targeted Delivery in ALA-PDT

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Keywords: Aminolevulinic acid; PDT; Peptide; Prodrug

Photodynamic therapy (PDT) is a minimally invasive approach for the treatment of cancer and various other human disorders, based on the selective activation of photosensitizers (PSs) with light. At present, one of the most promising strategies for PDT and also fluorescence photodiagnosis (PDD) is to use 5-aminolevulinic acid (ALA) as a prodrug to increase intracellular levels of the endogenous PS, protoporphyrin IX (PpIX). Although the clinical effectiveness of ALA-PDT has been widely demonstrated , the physicochemical properties and chemical reactivity of ALA still present some problems for future applications. An innovative way to address some of these challenges is to incorporate ALA units into a variety of prodrug systems,[1] and we have previously shown that peptide-based prodrugs are an attractive way to improve the delivery of ALA, leading to enhanced PpIX accumulation and PDT effects.[2] We present here our results on two classes of novel peptide-based prodrug systems designed to enhance the delivery of ALA to specific cell types. In one approach prodrugs are based on a molecular core to which multiple ALA units (ALA dendron derivatives) are attached as the effector units, and with ALA itself connected by an ester bond. The core structure is then linked to a variety of tumour-homing peptides via selective Cu-catalysed click chemistry. This combines the stategies of both ALA dendrimers and ALA-peptide prodrugs,[3] and as one proof of concept of this particular approach, we have prepared systems containing a bombesin-derived peptide that allows selective targeting of the GRP receptor (GRPR) which is overexpressed in a variety of tumours. In a second approach, ALA derivatives are embedded within a cyclic peptide prodrug structure which contains the targeting address within its seqeunce. Targeted ALA delivery and PpIX production with both types of prodrugs have been investigated in GRPR-expressing PC3 cells by fluorescence spectroscopy and confocal microscopy, and red and blue light-activated cell killing evaluated using cell viability (MTT) assays.

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Time-resolved Study of a C4h Phthalocyanine Interacting with Guanine-rich Nucleic Acid Sequences

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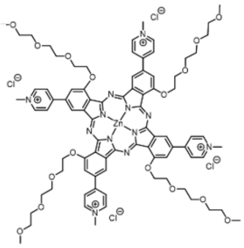
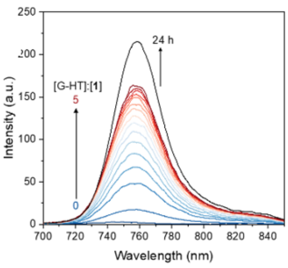
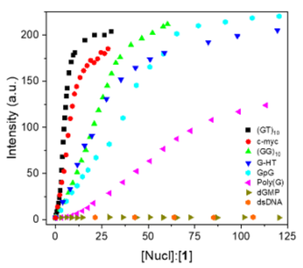
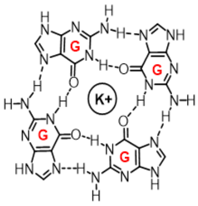
Phthalocyanines (Pcs) are highly effective photosensitizers for photodynamic therapy (PDT), owing to their strong absorption in the red to near-infrared region and high singlet oxygen quantum yields. Their performance can be further enhanced through careful molecular design, with many excellent phthalocyanine photosensitizers having been reported over the last few decades.[1] In this context, we have been developing synthetic strategies to access C_{4h}-symmetric Pcs such as **ZnPc1** to explore how molecular symmetry influences optical properties and interactions with biomolecules.[2] In aqueous solution, it is known that Pcs often aggregate, which may be particularly pronounced for regioregular compounds like **ZnPc1**. Aggregation quenches excited states, reducing PDT efficacy but potentially enabling an alternative photothermal mechanism of action.[3] We have investigated how G-rich nucleic acid sequences can disaggregate **ZnPc1** via sequence-specific interactions.[4] Interestingly, the disaggregation and binding occur with atypically slow kinetics, sometimes continuing over several hours. Our studies include sequences that form G-quadruplex structures, an important secondary-structure, such as in the oncogene *c*-MYC and human telomeres, with which strong interactions are observed. Using a combination of transient absorption and time-resolved infrared spectroscopy across femtosecond to millisecond timescales, we directly observed interactions between **ZnPc1** and G-bases, as evidenced by changes in carbonyl stretches upon Pc excitation. These techniques also allowed us to compare singlet- and triplet-state dynamics in monomeric, aggregated, and nucleic acid-bound forms. Our findings suggest that the complementary C_{4h} structure of **ZnPc1** and G-quadruplex structures, may facilitate specific and efficient binding.



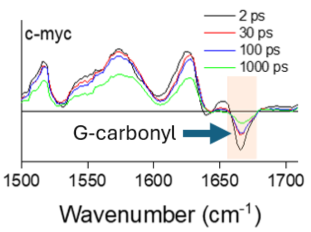
ZnPc1







G-quadruplex



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Non-Adiabatic Dynamics study on microhydrated DNA probe

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Keywords: Photochemistry, Non-Adiabatic dynamics, DNA probe.

The study of the native conformation of nucleic acids and its eventual perturbation induced by the coupling with external perturbation factors is fundamental to better assess the molecular mechanisms underlying their biological role.[1] Luminescent DNA probes [2] are tools of choice to evidence the presence of important structural modification of the nucleic acid structure finely and rapidly. I will present a non-adiabatic dynamics study, performed in the surface hopping formalism, on 2-thienyl-3-hydroxychromone, an environment-dependent luminescent organic DNA probe.[3] I will show that the first shell solvent water molecules undergo a rather complex reorganization upon light excitation.[4] This also involves the triggering of a water-mediated proton transfer process which leads to the formation of the tautomeric structure (Figure 1). The presence of this solvent-mediated transfer mechanism globally diminishes the intersystem crossing efficiency, and hence the population of the triplet state manifold, as compared to the non-solvated systems. The results also point out the non-innocent role of solvent networks in tuning complex photophysical processes, while opening competitive relaxation channels.

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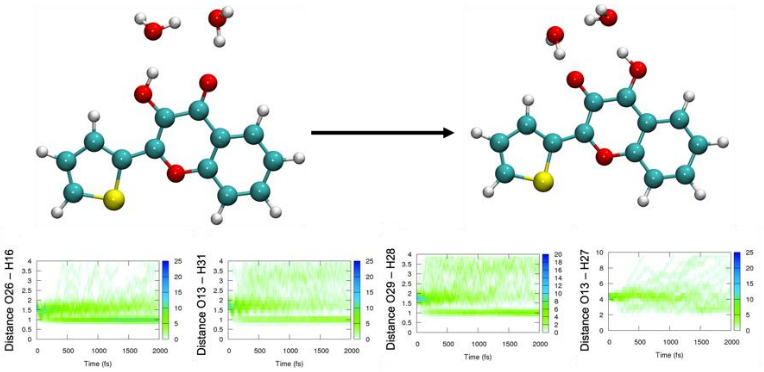

Figure 1. Tautomerization process

Oral communications on PDT
Chair: Amparo Faustino

A novel water-in-oil emulsion for photoimmunotherapy delivery

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Keywords: Photoimmunotherapy; hepatocellular carcinoma; water-in-oil emulsion;

Liver cancer ranks among the top five causes of cancer-related deaths in 90 countries, with global incidence projected to rise by 55% between 2020 and 2040 [1]. Hepatocellular carcinoma (HCC) accounts for approximately 90% of all liver cancers [2], yet around 80% of HCC patients are ineligible for curative treatments such as surgical resection or liver transplantation [3]. For these patients, locoregional therapies and systemic immunotherapies offer promising alternatives. However, while systemic immunotherapy has shown clinical benefits, it is often accompanied by significant adverse effects. This highlights the need for localized delivery strategies that can minimize treatment doses and reduce off-target toxicity. Photoimmunotherapy (PIT) is a targeted approach that combines a systemically administered photoimmunoconjugate (aka antibody-photosensitizer drug conjugate) with localized red-light exposure to selectively kill cancer cells and stimulate systemic anti-tumor immunity [4]. In this study, we developed a novel water-in-oil emulsion formulation to enable local delivery of photoimmunoconjugates directly to cancer cells, aiming to enhance PIT efficacy. This emulsion preserves the cancer-selectivity, singlet oxygen generation, and photoactivity of the photoimmunoconjugates, while significantly improving their uptake by cancer cells. Our approach provides a new method for delivering high payloads of photoimmunoconjugates to tumors locally, with the potential to reduce systemic toxicity and improve therapeutic outcomes.

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NIR-Responsive Plasmonic Nanoparticle-loaded Mesoporous Silica Nanostructures for Photo-Assisted Gram-Positive Bacteria Eradication

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Keywords: $Cu_{2-x}S$ nanoplates, mesoporous silica nanoparticles, photo-assisted therapy, antimicrobial effect

Implant-associated bone infection is increasingly emerging as a serious threat due to the high demand for orthopedic implants in our ageing society. Bacteria adhering to the surface of implants are highly resistant to conventional antibiotics and increasingly difficult to kill. In the quest for new antimicrobial strategies, multifunctional antimicrobial nanosystems offer new promise in the treatment of such infections. Herein, the development of mesoporous silica-coated plasmonic nanostructures for the delivery of antimicrobial drug for light-assisted therapy for bone infections is reported. Core@shell structures featuring $Cu_{2-x}S$ facet triangular nanoplates (NPL) core and a mesoporous silica shell ($Cu_{2-x}S$@MSS) were synthesized. Further loading with antimicrobial molecules, such as levofloxacin (Levo) o rifampicin (Rif), allowed to synergistically combine the $Cu_{2-x}S$ NPL inherent antibacterial photoactivity with the pharmacological effects of the drug. The silica-based nanostructures, synthesized using a microemulsion approach, were thoroughly characterized, and their antibacterial activity explored in terms of inhibition of bacteria growth against *Staphylococcus aureus*. These results outline future applications of these nanoformulations for the management of bone implant-associated bacterial infections [1].

This work was funded by "Nanocrystalline HYdroxyapatite for the LOcal DElivery of Anticancer agents in the treatment of bone tumors and metastases" NHYLODEA funded by the European Union - Next Generation EU, Mission 4 Component 1, CUP H53D23007980001 and Bilateral Project CNR-RFBR Russia Joint research project (2021-2023).

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Unveiling the Cell Death Mechanisms Induced by LUZ51-Mediated PDT

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Keywords: LUZ51-PDT, apoptosis, ferroptosis, paraptosis

Beyond apoptosis, emerging studies have identified other forms of regulated cell death, such as necroptosis, ferroptosis, pyroptosis, parthanatos, and paraptosis, with some of which have already been identified in PDT responses [1-2]. In this context, we are currently investigating the cell death pathways triggered by PDT when using a novel carboxamide halogenated bacteriochlorin, which offers significant advantages over clinically-approved photosensitizers due to its strong near-infrared (NIR) absorption ($\varepsilon_{743\ nm}$= 100,000 $M^{-1}.cm^{-1}$), high reactive oxygen species (ROS) quantum yield (Φ_Δ = 0.38), rapid cell internalization, and high phototoxicity [3].

PDT studies using LUZ51 in 4T1 mouse breast cancer cells showed that high concentrations of LUZ51 (200 nM, 1 J/cm^2) induced morphological changes typical of accidental necrosis, whereas lower concentrations (≤ 100 nM, 1 J/cm^2) resulted in chromatin condensation and fragmentation, membrane blebbing, and presence of apoptotic bodies, as observed by both optical and electron microscopy. In addition, the observation of phosphatidylserine exposure on the outer leaflet of the plasma membrane and caspase-3 activation shortly after irradiation further support the induction of apoptosis by LUZ51-based PDT.

When LUZ51-based PDT was performed in the presence of inhibitors targeting different cell death modalities - namely Z-VAD-FMK (apoptosis), necrostatin-1 (necroptosis) and, ferrostatin-1, liproxstatin-1 and deferoxamine (ferroptosis) - Z-VAD-FMK significantly delayed the progression of apoptosis, conferring a high level of protection at ~ 3 hours post-irradiation but not at 24hours. Interestingly, while both LUZ51-treated cells and those treated with LUZ51 + Z-VAD were progressively dying, they exhibited distinct morphological features, suggesting that alternative cell death mechanisms may be engaged when caspase activity is inhibited [2].

A protective effect was also observed with ferrostatin-1 and liproxstatin-1 (lipid ROS scavengers), which, similarly to Z-VAD, weas more evident at early time points after cell irradiation. At 24 hours post-irradiation, only minor differences in cell viability were detected. In contrast, deferoxamine (an iron chelator) failed to rescue cell viability upon LUZ51-based PDT. As expected, ferroptosis induced by RSL3 (used as positive control) was effectively inhibited not only by ferrostatin-1 and liproxstatin-1, but also by deferoxamine. Together, these results suggest that ferroptosis is not a major contributor to LUZ51-induced phototoxicity. The partial protection observed with ferrostatin-1 and liproxstatin-1 may instead result from their general antioxidant properties rather than from specific inhibition of ferroptosis. This interpretation is supported by a lipid peroxidation assays using BODIPY 581/591 C11, which revealed significant lipid oxidation, particularly at higher concentrations of LUZ51 (75 and 100 nM), an effect that was significantly reduced in the presence of liproxstatin-1.

Interestingly, apoptotic features were also observed in U2OS human osteosarcoma cells exposed to moderate PDT doses, whereas low PDT doses (e.g., 50 nM, 0.25 J/cm^2 or 12.5 nM, 1 J/cm^2) induced signs of paraptosis, characterized by cytoplasmic vacuolation, in U2OS but not in 4T1 cells. Fluorescence microscopy of U2OS cells co-overexpressing calreticulin-GFP—a protein localized to the endoplasmic reticulum (ER)—revealed that these vacuoles originated from the ER. The paraptotic response was significantly inhibited by cycloheximide (a protein synthesis inhibitor) as well as by SP600125 and SB202190, suggesting the involvement of JNK and p38 MAPK pathways, two major branches of the mitogen-activated protein kinase (MAPK), in LUZ51-mediated paraptosis in U2OS cells.

This project was funded by the Foundation for Science and Technology (UID/QUI/00313/2020 and PTDC/QUI-OUT/0303/2021).

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Oral communications on PDT
Chair: Amparo Faustino

Discovering the Potential of Amino-Based Flavylium Dyes for Photodynamic Therapy

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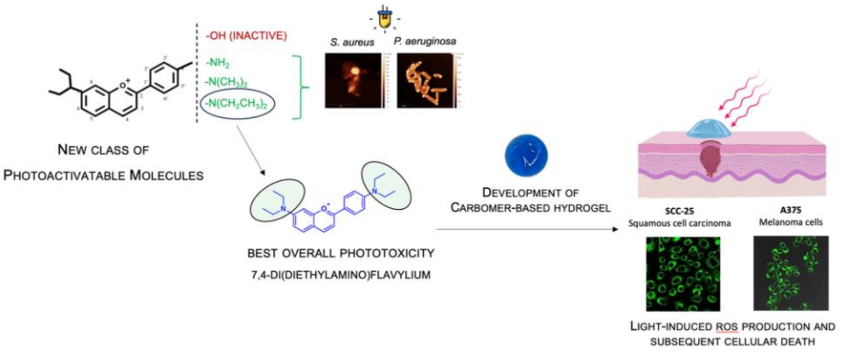
Keywords: Flavylium dyes; photodynamic therapy; topical treatment; carbomer-based gel;

ABSTRACT

Photodynamic therapy (PDT) is a minimally invasive therapeutic approach that has shown promising results in recent years, particularly in dermatology, in the treatment of bacterial infections, neoplastic disorders, and other abnormal skin conditions [1] [2]. This work explores the light-induced cytotoxicity of a group of synthetic amino-substituted flavylium dyes ($7NEt_24'NMe_2$,$7NEt_2st4'NMe_2$, $7NEt_24'NEt_2$ and $7NEt_24'NH_2$), recently proposed as a new class of photosensitizers. They showed strong capacity to mitigate bacterial growth (*S. aureus* and *P. aeruginosa*) in the presence of light (white, 22.5 J/cm^2), being particularly effective against the Gram + species, with bacterial growth being completely eradicated with concentrations in the range of 3–12 µM. Atomic force microscopy analysis confirmed substantial morphological changes after treatment and singlet oxygen was shown to be the primary driver of the photodamage observed. Moreover, the most active flavylium, 7,4-di(diethylamino)flavylium, was further evaluated as a potential photosensitizer in the treatment of skin lesions. Irradiated squamous carcinoma melanoma cells in the presence of 0.75 µM flavylium caused more than 80% cell viability reduction. Physical and chemical properties of the flavylium were also tested when incorporated in formulations for topical delivery. The carbomer-based gel provided interesting absorption and fluorescence increases, combined with a noteworthy photostability (~ 98%) after light exposure of 90 J/cm^2, proving to be a suitable formulation for PDT-related applications.



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Ru-COUBPY COMPLEXES AS POTENT PHOTOSENSITIZERS FOR CANCER PHOTOTHERAPY

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Keywords: Ruthenium complex; Photosensitizer; Coumarin; PDT

Photodynamic therapy (PDT) is a well-established, non-invasive cancer treatment modality that relies on the generation of a variety of cytotoxic reactive oxygen species (ROS) through the irradiation of a photosensitizer drug (PS) that induce tumor cell death. However, the efficacy of most marketed PSs based on the tetrapyrrolic scaffold is often compromised when treating large hypoxic solid tumors due to its oxygen dependence and the limited tissue penetration of short wavelength light. To overcome these challenges, we have developed a novel family of potent PSs based on Ru(II) polypyridyl complexes incorporating coumarin-based COUBPY ligands exhibiting outstanding *in vitro* and *in vivo* PDT efficacy when irradiated with light within the phototherapeutic window.[1]

Here we introduce a second generation of Ru-COUBPY PSs with enhanced NIR absorption through strategic modifications of the coumarin ligand. These new PSs photogenerate ROS via both Type I and Type II mechanisms and exhibit strong phototoxicity against cancer cells upon irradiation with light within the phototherapeutic window, including NIR light. Furthermore, *in vivo* PDT efficacy studies with a Ru-COUBPY PS demonstrated a potent tumor growth inhibition in a subcutaneous CT-26 tumor model upon irradiation with NIR light at 780 nm.

Acknowledgements: This work was supported by funds from the Spanish Ministerio de Ciencia e Innovación-Agencia Estatal de Investigación (MCI/AEI/10.13039/501100011033) and FEDER funds (PID2020-117508RB-I00 and PID2023-146161OB-I00), the Departament de Recerca i Universitats de la Generalitat de Catalunya (2021 LLAV 00083, 2024 INNOV 00002, 2024 PROD 00005), the Fundació Bosch i Gimpera (F2I-PdC_2022-007) and ”la Caixa” Foundation under the Grant LCF/TR/CI23/56000013.

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Oral communications on PDT
Chair: Amparo Faustino

Metallochlorins for photodynamic therapy: Experimental results and theoretical calculations

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Keywords: synthesis; density functional theory calculations; photosensitizer activity; singlet oxygen generators

Photodynamic therapy (PDT) is a therapeutic modality that combines three essential components: light, molecular oxygen, and a photosensitizer, to generate reactive oxygen species (ROS) capable of inducing cellular damage. This approach has diverse applications in medical fields, particularly in cancer treatment and combating infectious diseases. Recent advancements in PDT have emphasized the development of novel photosensitizers and the genetic engineering of biological photosensitizers to enhance ROS production and explore cellular signaling pathways [1].

In this study, we synthesized and characterized a series of metallochlorins, providing insights into their ability to generate singlet oxygen for therapeutic applications (Fig. 1). We aimed to investigate the influence of metal ions, including Zn(II), Cu(II), Fe(III), Pd(II), Pt(II), Pt(IV), Os(II), Ru(II), Ir(III) and Sn(IV), on chlorin structure and properties, particularly regarding the Q band in electronic spectra, the spin-orbit coupling efficiency and triplet state stability, which are critical for determining the efficacy of a photosensitizer. The synthetic methodology employed 1,3-dipolar cycloadditions and metallations, using conventional, microwave and/or ohmic heating [2, 3]. The advantages and limitations of these heating methods will be discussed.

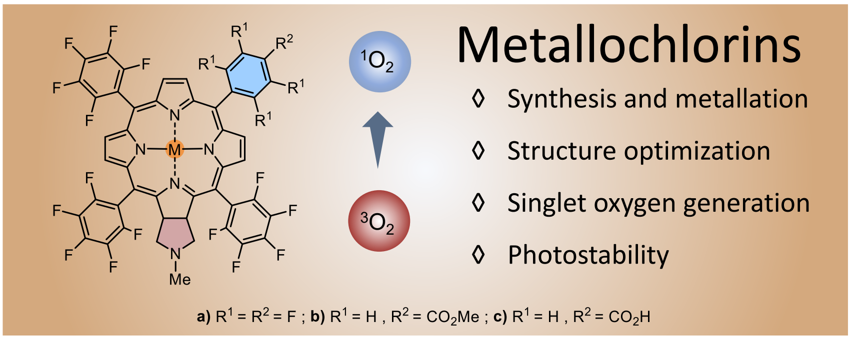

Fig. 1 – Structure of metallochlorins aiming PDT

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Molybdenum Clusters for Enhanced Photodynamic and X-ray-Induced Cancer Therapy

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Keywords: X-PDT; Molybdenum clusters; Cancer therapy, Nanomedicine

Photodynamic therapy (PDT) represents a promising approach to cancer treatment, leveraging the cytotoxic potential of reactive oxygen species generated upon activation of photosensitizers by light. However, the limited penetration of light into deep tissues remains a critical barrier to the broader application of PDT in oncology. In response, X-ray-induced photodynamic therapy (X-PDT) has emerged as a complementary strategy, enabling the activation of radiosensitizers by ionising radiation and extending the therapeutic reach to tumours that were previously inaccessible.

This study focuses on the development and optimisation of octahedral molybdenum cluster-based formulations for use in PDT and X-PDT. The goal is to enhance the therapeutic efficiency and selectivity of these photo/radiosensitizers through ligand exchange, followed by nanoformulation, thereby addressing the challenges of light penetrability and systemic toxicity in conventional photodynamic therapy (PDT)[1]. Molybdenum clusters with the $\{Mo_6I_8\}^{4+}$ core were synthesised and formulated into nanoparticles. *In vitro* experiments on cancer cell lines demonstrated that they exhibit strong phototoxicity under 460 nm light irradiation, with IC_{50} values as low as 40 nM and no detectable toxicity in the dark (>50 μM).[2] Upon X-ray exposure, cells treated with nanoparticles exhibit a significantly reduced clonogenic potential, with a dose enhancement factor of approximately 1.9, resulting in a lower dose necessary to achieve the desired antiproliferative effect. Treated cells showed a marked increase in intracellular reactive oxygen species, consistent with oxidative stress-mediated cytotoxicity. Importantly, the subcellular distribution of the clusters—targeting mitochondria, lysosomes, or the plasma membrane—was strongly influenced by their chemical structure.[1, 2, 4] The cell death mode pathway is beginning to exhibit characteristics distinct from the classical processes of apoptosis and necrosis, suggesting a non-canonical mode, such as ferroptosis[3]. Octahedral molybdenum clusters represent a promising dual-function therapeutic platform for both PDT and X-PDT. Their structural versatility and ability to be activated by both visible light and X-rays make them suitable candidates for treating deep-seated tumours. Targeted formulations, such as HA-cluster conjugates, could improve bioavailability and specificity, thereby further enhancing their clinical potential. The ultimate goal of this research is to validate these findings in *in vivo* models and advance toward clinically relevant, integrated therapeutic strategies. This work was supported from the grant of Specific university research – grant No A2_FPBT_2025_053.

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Photoprotection and Climate Change

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Keywords: photoprotection, climate change, pollution, skin cancer.

Climate change poses emerging challenges for dermatology due to its multifaceted impact on skin health. Rising ambient temperatures, increased UV radiation, and higher levels of air pollution contribute to a growing incidence of skin diseases, including infections, inflammatory conditions, and cutaneous malignancies. Solar irradiance, heat index, and pollution have a direct effect on skin ageing and cancer risk, supported by epidemiological and exposomal studies in diverse populations.

The damaging mechanisms of pollutants and UVR include oxidative stress and skin barrier disruption, highlighting the synergistic effect of pollution and ultraviolet radiation. Vulnerable populations, including individuals with xeroderma pigmentosum, albinism, and outdoor workers, require targeted photoprotection strategies.

Innovative approaches include the use of phototype-adapted sunscreens, antioxidants, cooling hydrogels, and mobile technologies for personalized UV and temperature monitoring.

In conclusión, a shift from conventional sun protection to a broader concept of *Environmental Protection*, incorporating temperature, humidity, and pollution, especially in at-risk groups is needed. Dermatologists are called to lead efforts in patient education, sustainable practice, and climate-health advocacy.

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The role of the microbiome on sunlight effects and photoprotection

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The human skin's microbiome hosts innumerable microorganisms and maintains homeostasis with the immune system. Ultraviolet radiation (UVR) hits the microbiome on the surface of the skin before reaching any cells in the body. Therefore, it is not surprising that UVR, with its bactericidal effects, may affect homeostasis in health and disease. Furthermore, UVR can indirectly influence the skin's microbiome by inducing antimicrobial peptides (AMPs), cis-urocanic acid (UCA) and other molecules. For example, UV-induced cis-UCA has been shown to affect the growth and diversity of the microbiota. Certain bacteria metabolize cis-UCA, a well-known immune modulator; therefore, eliminating these bacteria may affect the immune response to UVR exposure. Experimental studies using germ-free or disinfected mice have shown that an intact skin microbiome has overall immunoprotective properties. Studies of human patients with polymorphic light eruption have indicated that eliminating skin microbiota through disinfection can restore impaired cytokine production and release upon UV exposure. Taken together, these findings suggest that topical modulation of the skin's microbiome may have ambivalent effects on immune function after UV exposure. On the one hand, it could be beneficial in certain diseases, such as PLE, atopic dermatitis, and cutaneous T-cell lymphoma. However, it could also favor UV-induced carcinogenesis. A better understanding of these relationships could lead to the development of new preventive and/or therapeutic strategies, such as targeted interventions to modulate the skin microbiome in health and disease.

GONZALEZ

Nuclear DNA Lesions Drive Metabolic Reprogramming in Keratinocytes Following Acute UVB Exposure

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Abstract:

Ultraviolet B (UVB) radiation from sunlight is a primary environmental factor responsible for DNA damage and the onset of skin carcinogenesis. DNA damage response (DDR) involves sensing the damage and subsequently signaling to downstream effectors. This network includes different cell signaling pathways, such as DNA repair systems or apoptosis, which are activated to maintain cellular homeostasis in responses to a stressor. However, the contribution of energy metabolism to this process remains underexplored. In this study, we investigated how nuclear and mitochondrial cyclobutane pyrimidine dimers (CPDs) affect metabolic pathways in UVB-exposed keratinocytes. To this end, we took advantage of keratinocytes expressing nuclear or mitochondrial CPD photolyases. Applying comprehensive proteomic analysis and metabolomics, we found a biphasic alteration in numerous metabolic pathways (such as glycolysis, oxidative phosphorylation, fatty acid B-oxidation) after acute exposure of keratinocyte to UVB radiation, which was largely dependent on nuclear DNA damage. Using live imaging and bioinformatic analyses, we found that the structure of the mitochondrial network was dynamically modified after UVB irradiation and those modifications were affected by both mitochondrial and nuclear DNA damage.

Altogether, our findings revealed dynamic, biphasic changes in metabolite profiles and metabolic fluxes, with a significant dependence on nuclear CPDs. Additionally, enhanced oxygen consumption and mitochondrial remodeling were found to be influenced by both nuclear and mitochondrial DNA damage. These results highlight the pivotal role of DNA damage location in orchestrating metabolic responses to UVB stress, offering novel insights into mechanisms underlying skin cancer development and potential therapeutic avenues.

Evaluating Photoprotective Properties of UK Garments with Integrating-Sphere Spectrophotometry

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Keywords: Ultraviolet radiation (UVR); Ultraviolet protection factor (UPF); Percentage visible light protection factor (pVL-PF); Protoporphyrim IX protection factor (PpIX-PF).

Introduction: Clothing can be a highly effective barrier against photodamage from UVR and visible light (VL). The UPF is the standard metric used to characterize a garment's defence against erythemal UVR, while the in-vitro pVL-PF and the in-vitro PpIX-PF are less commonly used to describe protection from blue light and PpIX activating wavelengths respectively [1-4]. These indices can be important for people with heightened sensitivity to light, whose skin may react to lower doses of UVR or VL than non-sensitive individuals. This study aims to evaluate the photoprotective properties of everyday clothing to better inform recommendations for people with photosensitivity.

Method: Multiple garments were purchased from UK retailers, including four black t-shirts, two navy t-shirts, a pair of black leggings, a pair of black cycling shorts, and a pair of black trousers. Fabric samples, 5 cm x 5 cm, were cut from two distinct areas of each garment. Transmission and reflection of each sample was measured from 290-800 nm in 1 nm increments using a spectrophotometer with integrating sphere (JASCO 60mm UV-Visible/NIR). Each sample was measured three times and the average transmittance value was used to calculate the UPF using the CIE erythema action spectrum [5]. Similarly, PpIX-PF was determined using the PpIX action spectrum from 350-800 nm, and pVL-PF was calculated from 400-469 nm.

Results: The initial five samples exhibited UPF values consistent with those reported by independent colleagues at the UK Health Security Agency, validating the measurement technique. A pair of black leggings provided the highest UPF of 2,048 (303 to 3,793, 95% confidence interval) whilst the lowest UPF was a navy t-shirt at 59 (36 to 82). Similarly, the black leggings provided the highest PpIX-PF and pVL-PF with 1238 (427 to 2047) and 99.9% (99.82% to 99.98%) respectively. Whilst the navy t-shirt provided the lowest with 48 (31 to 65) PpIX-PF and 97.8% (97.1% to 98.5%) pVL-PF.

Discussion: These findings indicate that, prior to wearing and washing, everyday garments can offer high levels of photoprotection. Variability in fabric, colour and weave appear to influence the degree of protection. This study underscores the potential for people with photosensitivity to benefit from a wider array of clothing options beyond those explicitly labelled with UPF ratings for effective sun protection.

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Vitamin D Receptor and Oxidative Stress

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Keywords: Vitamin D Recepto;. Oxidative stress; Photoprotection; *Aspalathus linearis; Polypodium leucotomos*

Vitamin D (VD), through its receptor (VDR), plays an essential role in the skin by regulating keratinocyte proliferation and differentiation, promoting DNA repair, and modulating the antioxidant response. Sun exposure, particularly to ultraviolet B radiation, is necessary for the cutaneous synthesis of VD, and appears to contribute to skin protection. However, some studies suggest that sun exposure itself and oxidative stress may influence VDR levels in skin cells, in addition to increasing the risk of oncogenic mutations that could lead to skin cancer [1–2]. This controversy limits the ability to provide clear recommendations regarding VD supplementation or photoprotection strategies, highlighting the need for more rigorous research. In this context, our study aims to develop an experimental model to evaluate oxidative stress-induced damage and to study VDR expression levels in skin cells and tissues. In this model, we also assess the protective abilities of a novel botanical formulation derived from the combination of two different *Aspalathus linearis* extracts and a specific extract from the leaves of *Polypodium leucotomos*, Aspa-Fernblock®. Using HaCaT cell line and ex-vivo epidermal equivalents, we subjected the models to different doses of hydrogen peroxide (H_2O_2) to mimic UV-induced oxidative damage. VDR levels, nuclear factor erythroid 2-related factor 2 (NRF2, a key regulator of antioxidant responses), and cell viability were assessed. Our results showed that in both models, H_2O_2 treatment induced a significant increase in cell death, as expected, but also a significant reduction in VDR expression in the viable cells, as well as an increase in nuclear NRF2 levels. We can then conclude that VDR expression levels are reduced following oxidative damage. Additionaly, using the mentioned experimental models, pretreatment with Aspa-Fernblock® was effective in protecting the levels of vitamin D receptors under oxidative stress conditions in an ex vivo human skin models. Therefore, Aspa-Fernblock®, shows promise as a photoprotective technology that preserves VDR in the skin subjected to oxidative stress, being an excellent, safe, and effective alternative strategy for photoprotection.

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Urinary Thymidine Dimer Excretion in Tanning Bed Users Compared with Sun Exposure During a Danish Summer

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Keywords: Ultraviolet radiation; Thymidine dimers; Tanning beds; DNA damage

Ultraviolet radiation (UVR) is a key environmental carcinogen that induces the formation of DNA damage such as cyclobutane pyrimidine dimers (CPDs), notably thymidine dimers (T<>T), which serve as biomarkers of UVR exposure. We present findings from two complementary studies investigating urinary excretion of repaired T<>T in healthy Danish populations exposed to UVR from commercial tanning beds in winter and natural sunlight in summer.

In the first study, 19 tanning bed users were monitored during winter using personal UVR dosimeters (SunSavers) and collected daily urine samples over four weeks. Urinary T<>T levels were quantified using a recently developed UPLC-MS/MS method [1,2] and were detectable in all 19 participants. The mean UVR dose was 1.52 SED per session (range: 0.28–3.95 SED), and users excreted an average of 857 ng T<>T/day. The users were using tanning beds at least twice a week and had an average of 94% body surface area exposed.

In the second study, 59 healthy individuals wore SunSaver dosimeters over 12 summer weeks. The mean daily UVR dose was 1.46 SED, with a mean T<>T excretion of 437 ng/day and an average of 46% body surface area exposed. Over the study period, the cumulative mean personal exposure reached 111 SED, with peak T<>T excretion measured one week after peak UVR exposure (mid-July). Younger participants exhibited significantly higher T<>T levels per unit UVR compared with older individuals, suggesting an age-related decline in DNA repair efficiency. As expected, individuals with darker skin types excreted significantly fewer T<>T dimers than those with lighter skin. Sex also influenced the correlation between UVR exposure and T<>T excretion, with male participants excreting higher levels than females under similar UVR conditions—possibly reflecting differences in sun protection behavior.

These findings underscore the intense and extensive UVR exposure associated with tanning bed use, characterized by high SEDs and nearly full-body exposure, resulting in almost double the urinary T<>T excretion compared with the summer cohort. The results highlight the photobiological risks of tanning beds, and support the use of urinary T<>T as a sensitive, non-invasive biomarker for assessing personal UVR exposure.

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Wednesday, August 27th

Photocatalytic water oxidation for oxygen-independent PDT

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Keywords: Photoredox catalysis, hydroxyl radicals, visible light, small molecule

Local hypoxia, a pervasive feature of solid tumours, dramatically impairs oxygen-dependent generation of ROS, leading to the failure of PDT [1,2]. How to effectively tackle hypoxia in PDT has spurred worldwide interest since most PSs act via oxygen-dependent mechanisms. One approach is to ensure an adequate oxygen supply during the illumination phase of PDT [3]. Photoredox catalysis has recently emerged as a more competent anticancer strategy for oxygen-poor environments [4]. Photocatalysts with an electron donor–acceptor type molecular structure can promote electron transfer to/from suitable acceptors/donors [5]. This molecular design has been explored as an effective strategy to obtain PSs with high oxidation/reduction potential that enables substrate oxidation/reduction from their excited state [4-6]. However, the success of this strategy relies on the proximity of the PS and the biological substrates, which limits its efficacy to their local distribution and depletion. In contrast, water is the most abundant and ubiquitous molecule in cells and thus represents an ideal substrate for the efficient generation of ROS for PDT through photocatalytic oxidation process. Recently, a donor-acceptor molecular system capable of producing hydroxyl radicals *via* water oxidation through a double-electron transfer process has been reported [7]. We show in this work that it is possible to further streamline water photooxidation using a small molecule and visible-light to produce hydroxyl radicals in a single proton-coupled electron-transfer step. We further demonstrate that this photochemical strategy is effective for inducing cell death under extreme hypoxia conditions. Small molecule, single electron transfer photocatalytic water oxidation may become the next game changer in photodynamic therapy, freeing it at last from its long-standing oxygen dependence limitation.

Funding: This work was supported by funds from the Spanish Ministerio de Ciencia e Innovación-Agencia Estatal de Investigación (MCI/AEI/10.13039/501100011033) and FEDER (Projects PID2020-115801RB-C22 and PID2023-149483NB-C22). S.N. thanks the Departament de Recerca i Universitats de la Generalitat de Catalunya for the support given to our research group (2021 SGR 01023) and the ICREA-Catalan Institution for Research and Advanced Studies for grant No. Ac2232308. R.B.O. thanks the Spanish Agencia Estatal de Investigación for a Ramon y Cajal contract (RYC2021-032773-I)

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ROS dosimetry and effect of oxygen consumption on PDT efficacy

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Keywords: ROSED; PDT; fractionated PDT; Efficacy

Photodynamic therapy (PDT) is used for cancer treatment based on the interaction of a photosensitizer (PS), light, and oxygen. The photodynamic interaction is termed type I and II depending on whether the cytotoxic oxygen species is through an electron transfer, producing oxygen superoxide and its secondary reactive oxygen species (ROS) or an energy transfer, producing singlet oxygen (${}^1O_2$). Most photosensitizers exhibit type II interactions. This talk explains quantitative dosimetry of ${}^1O_2$ via either singlet oxygen explicit dosimetry (SOED) using explicit dosimetry of light fluence rate (ϕ), PS concentration ([PS]), and oxygen concentration ($[{}^3O_2]$) or singlet oxygen luminescence dosimetry (SOLD). Secondly, the SOED model has been applied to several type II (e.g., HPPH, BPD, Photofrin, and prodrug ALA) photosensitizers. Cure index was computed from the rate of tumor regrowth after treatment and was compared against three calculated dose metrics: total light fluence, PDT dose (product of light fluence and PS concentration), and reacted $[ROS]_{rx}$, demonstrating that $[ROS]_{rx}$ serves as a better dosimetric quantity for predicting treatment outcome. Finaly, fractionation PDT treatment has shown to significantly improve long-term local control (LCR at 90 days) in vivo resulting significant decrease of threshold dose of $[ROS]_{rx}$ for type II interactions.

Selective targeting of proteins and pathways using photopharmacology

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Keywords: Photopharmacology, photodynamic therapy, metal homeostasis, protein homeostasis, enzyme inhibitors

EXTENDED ABSTRACT

Phototherapy approaches include photodynamic therapy (PDT), which utilizes chemically stable photocatalysts to sensitize the conversion of endogenous molecules such as oxygen to form transient reactive species (ROS/RNS), and photopharmacology, a complementary approach that relies on molecules that undergo self-modifying photochemistry, such as bond cleavage reactions for the delivery of biologically active products. Ru(II) polypyridyl systems have demonstrated utility for both approaches, and Ru(II) PDT is now well validated, with exceptional patient outcomes from the use of TLD-1433 treatment for bladder cancer. Photopharmacology approaches are at an earlier stage of development, but have great potential to achieve selective control over the activity of specific enzymes and protein signaling pathways. The one key limitation of photopharmacology is the fact that it is a stoichiometric approach, in contrast to the photocatalytic nature of PDT, which imparts exceptional activities from very low concentrations of photoactive species. However, an advantage is that photopharmacology generally relies on oxygen-independent processes, and thus can be applied in hypoxia We have developed Ru(II) photocages to deliver small molecules ranging from enzyme inhibitors to Proteolysis Targeting Chimeras (PROTACs). Our current focus is on photocontrol over transcription factors and immunomodulators in order to impact key pathways in cancer progression and immune regulation. A second focus is on copper homeostasis, as dysregulation of copper is associated with neurological disorders, malignant progression, and immune response. We have created new agents that put both chemical and biological regulation of copper trafficking and sequestration under the control of light. These strategies take advantage of intrinsic biological amplification processes, and are anticipated to provide potent synergy with other treatment approaches, including PDT, and are amenable to the creation of multifaceted light-activated systems. The new molecular probes and potential therapeutics are expected to generate novel insights into cellular signaling processes, including fast studies of dynamic events, and to provide a path towards the controlled regulation of immune response.

Unconventional Reactive Oxygen Generation and Biological Outcomes

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Keywords: Water Oxidation, Spin-Flip Electron Transfer, Pyroptosis, Oxygen-Confined Photooxidation

EXTENDED ABSTRACT

Reactive oxygen species (ROS, $^1O_2$, $O_2^{\cdot}$, OH·, and H_2O_2) are widely recognized for their dual roles in biological systems, contributing to both cellular signaling and oxidative stress-induced damage. While conventional ROS generation pathways have been extensively explored, emerging evidence highlights unconventional mechanism of ROS generation and their non-canonical biological consequences.

In this presentation, I will deliver three recent studies that unveil novel aspects of photoinduced ROS generation and their biological outcomes. First, rational design strategy for ROS generation under hypoxic condition will be discussed, achieved via water photooxidation.[1] This oxidative photocatalysis at cellular membranes can initiate non-canonical pyroptosis, a unique form of inflammatory cell death distinct from classical pathway. Notably, in-vivo mouse models confirmed the introduction of immunogenic cell death. In the next study, a spin-flip based electron transfer mechanism is uncovered, enabling efficient singlet oxygen generation from polyaminoglycerol, bypassing the traditional Type II energy transfer mechanism, and leading to potent antibacterial effect.[2] This finding paves the way for the design of biocompatible aliphatic organic photosensitizers. Finally, this unconventional singlet oxygen generation also contributes to revealing a hidden route of protein damage through oxygen-confined photooxidation within microenvironment, emphasizing how spatially restrict ROS can induce site-selective biomolecular damage.[3] Such photooxidative mechanism may represent a latent oxidation pathway in human tissues directly exposed to visible light, such as skin and eyes.

Together, these works highlight the importance of revisiting ROS generation beyond classical paradigms and understanding their spatial, mechanistic, and biological specificity. This presentation will provide an integrative view of these unconventional ROS pathways and discuss their implications in cell death, immunogenicity, therapeutic applications, and protein damages under visible light.

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BODIPYs as Photodynamic and Photothermal Agents with Unprecedented Phototoxic Indexes under Normoxic and Hypoxic Conditions

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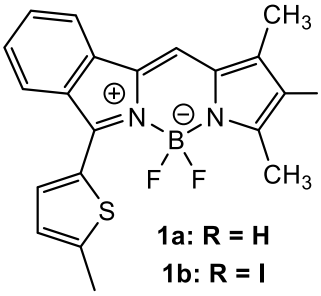
Keywords: PDT, PTT, aggregation, irradiation

Background and Objective

We wanted to find out how effective in killing cancer cells our novel BODIPY photosensitizer (**1a** and **1b**, see figure on the right) were under normoxic and hypoxic conditions.



1a: R = H
1b: R = I

Materials and Methods

The BODIPY photosensitizer were successfully synthesized. Their dark and phototoxicity were determined against various cancerous and non-cancerous cell lines under normoxic and hypoxic conditions.[1]

Results and Discussion

The presented compounds (**1a** and **1b**) show extremely high toxicity upon irradiation with light and low dark toxicity in different cancer cell lines in 2D culture as well as in 3D multicellular tumour spheroids (MCTSs). The ratio of dark to light toxicity (phototoxic index, PI [2,3]) reaches values exceeding 830’000 after irradiation with energetically low doses of light at 630 nm (5.0 J/cm^2). Under hypoxic conditions (0.2% O_2), a striking PI of 360’000 was observed. To the best of our knowledge, both PI values are the highest reported to date.

We have strong evidence for the following mechanisms of action (MoA): apart from the classical photodynamic action with singlet oxygen, especially compound **1a** and both compounds under hypoxic conditions exert their phototoxicity via an aggregation based photothermal mechanism.

Conclusions

The oxygen-dependent mechanism of action of established PSs hampers effective clinical deployment. We anticipate that small molecule-based agents with a photothermal MOA, such as the presented BODIPY-based compounds **1a** and **1b**, may overcome this barrier and provide a new avenue to cancer therapy.

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Nature's Photosensitizers: What They Are, Where They Hide, and How to Discover Them

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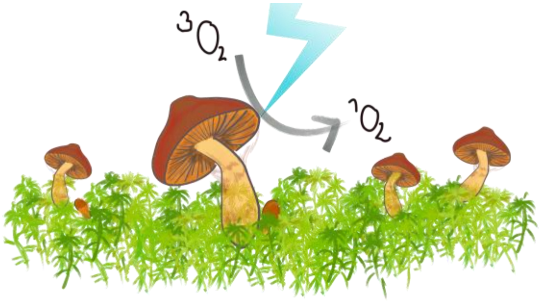
Keywords: Natural Photosensitizers, Fungal Photosensitizer, Anthraquinones, Photoactivated Defense

Sessile organisms have evolved a diverse array of defense strategies shaped by co-evolution with predators and competitors. Among these, photobiological defense strategies –where light triggers specific biochemical responses– are of particular interest. To avoid self-inflicted damage, these organisms protect themselves by compartmentalizing photosensitizers in roots or specialized organelles, deactivating them through redox reactions, the formation of labile precursors, or by combining them with antioxidants. While a handful of natural products capable of generating reactive species for photodynamic inhibition and therapy have transitioned, in native or semi-synthetic forms, into clinical use, the vast majority remain obscure: some briefly discovered and recognized, many overlooked, and most quickly forgotten.



Here, we delve into the world of photo-activatable natural compounds, examining their chemical structures, cellular localizations, and strategies for isolation. A special focus will be laid on fungi and their photochemical and photopharmacological properties revealed in course of research projects over the past years. Through this exploration, we highlight both the extraordinary diversity and potential of these natural products, as well as the significant gaps that remain in our understanding.

Acknowledgement:
Funding of the Austrian Science Fund for the Projects PhotoFungal (10.55776/P31915), POSADEC (10.55776/I5867), and PenTaPho (10.55776/P37163) is highly acknowledged.

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Natural Dye Extracts as Photosensitizers for Sustainable Antimicrobial Applications

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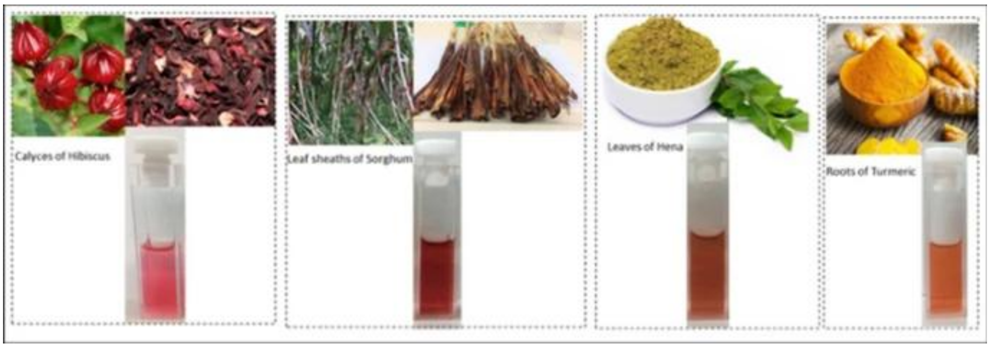
Keywords: Photodynamic inactivation, natural photosensitizers, food-grade disinfection

EXTENDED ABSTRACT

This study explores the antimicrobial potential of crude methanolic dye extracts from four non-staple plants—*Lawsonia inermis*, *Hibiscus sabdariffa*, *Curcuma longa*, and *Sorghum bicolor*—as natural photosensitizers [1,2,3]. Their photostability and efficacy were evaluated under visible light and dark conditions against bacteria (*E. coli*, *B. subtilis*) and surrogate viruses (MS2, phi6) in different aqueous media. *Curcuma longa* showed the strongest photodynamic activity, while *L. inermis* demonstrated broad-spectrum antimicrobial action even in the absence of light, especially in acidic or aqueous solutions. *H. sabdariffa* extracts were particularly effective in water, supporting their use in low-tech, sunlight-driven sanitation of fresh produce.



These findings position these natural dyes as promising, low-cost, eco-friendly alternatives for disinfection in food safety and healthcare, particularly in resource-limited settings.

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Fungal Photosensitizers: Dual Potential for Photodynamic Antimicrobial and Cancer Applications?

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Keywords: Photodynamic Therapy; OSMAC approach; *Talaromyces*, *Penicillium*

EXTENDED ABSTRACT

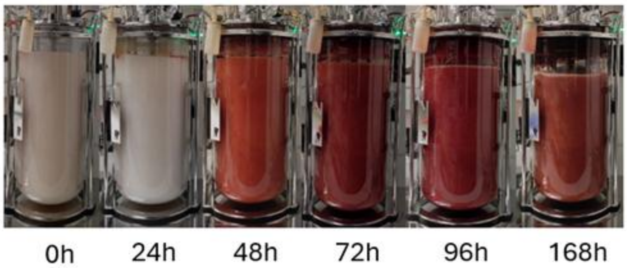
Fungal biotechnology offers a sustainable and scalable platform for producing high-value secondary metabolites, including photosensitizers (PS) with significant biomedical potential. In this study, we aim to develop and implement a robust screening workflow to discover and biotechnologically exploit PS from *Penicillium* and *Talaromyces* species, targeting both photoantimicrobial and photocytotoxic activities.

Using the OSMAC (One Strain, Many Compounds) strategy, we systematically varied cultivation parameters (e.g., media composition, temperature, light exposure, co-cultivation) to enhance the chemodiversity and yield of bioactive metabolites. Both solid and submerged (including bioreactor) cultures were employed, with a particular emphasis on conditions for potential industrial-scale production. Crude extracts were screened for photocytotoxicity against cancer cell lines (i.e., nasopharynx, bladder, hypopharynx, esophageal, and nasal septum) and for photoantimicrobial activity against *Escherichia coli*, *Staphylococcus aureus,* and *Candida albicans*. UHPLC-HRMS/MS analysis enabled dereplication and putative annotation of bioactive metabolites.

To date, several species—including *Talaromyces wortmannii, T. islandicus*, *T. stipidatus*, and *Penicillium restrictum*—have been identified as producers of potent PS. The production and detectability of these PS were found to be highly dependent on cultivation conditions, with some compounds occasionally masked by co-occurring mycotoxins during screening. Notably, some tested fungi exhibited dual bioactivity: for example, under blue light irradiation, extracts from *T. islandicus* and *T. stipidatus* showed light-dependent cytotoxicity across all tested cancer cell lines, and also demonstrated photoantimicrobial activity against *S. aureus* (both) or *C. albicans* (*T. islandicus*). Initial attempts to trigger pigment production in *T. islandicus* in bioreactor cultures were successful (Fig). Our findings underline that filamentous fungi are a promising and renewable source of PS for photodynamic applications in both antimicrobial and anticancer therapy.

This work was supported by the Austrian Science Fund (P37163).

Figure. Pigment accumulation in *T. islandicus* grown for 7 days in a bioreactor batch culture at 30°C on rice-based medium.



Photoinactivation of *Trypanosoma cruzi:* effects on lipids and proteins.

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Keywords: Photonactivation; *T. cruzi*; Curcumin; AFM; DSC.

ABSTRACT

Photoinactivation is a procedure to eradicate pathogens, such as bacteria, virus, fungus, and parasites. In this work, it was applied to *T. cruzi* epimastigotes cultures (strain CL Brener). The structural modifications in lipids, proteins, and nucleic acids in the parasites were investigated after the addition of curcumin and blue light radiation. Atomic Force Microscope (AFM) and Differential Scanning Calorimetry (DSC) measurements were obtained. The results showed that curcumin produces a citotoxic effect on epimastigotes depending on its concentration. Photoinactivation during one hour with 450 nm radiation diminishes the total *T. cruzi* viability inducing morphological changes in the parasites. This is because the denaturation of proteins. Stiffening of the lipid membrane was observed presumably due to lipid peroxidation [1]. Electrophoresis did not show any DNA degradation in the photo-inactivated parasites. Because the above event is not immediate, it would be interesting to evaluate DNA integrity some hours after the photoinactivation process, since fragmentation has been detected when evaluating DNA 24 hours after the procedure [2].

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On Novel Angular Furocoumarins and Their Interaction With DNA

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Keywords: Angelicins; Time-Resolved Spectroscopy; Photoinduced Electron Transfer; Photoaddition

Angelicins (angular, Fig. 1a) and psoralens (linear) are furocoumarins found in various plants, where they act as phytoalexins. Psoralens are well-known for their application in PUVA (psoralen + UV-A irradiation) therapy of autoimmune skin disorders. Angelicins have recently gained interest due to their distinct photobiological properties [1]. While psoralens intercalate reversibly into DNA and form photoadducts (monoadducts, followed by interstrand cross-links) exclusively with thymine (T), they also undergo ultrafast photoinduced electron transfer (PET) with guanine (G), limiting cytotoxic efficacy [2]. Angelicins, however, form monoadducts with T (Fig. 1b) *and* cytosine (C; Fig. 1c) bases without inducing interstrand cross-links, which may reduce phototoxicity [3]. Despite their biomedical potential, there is a lack of spectroscopic studies on angelicins.

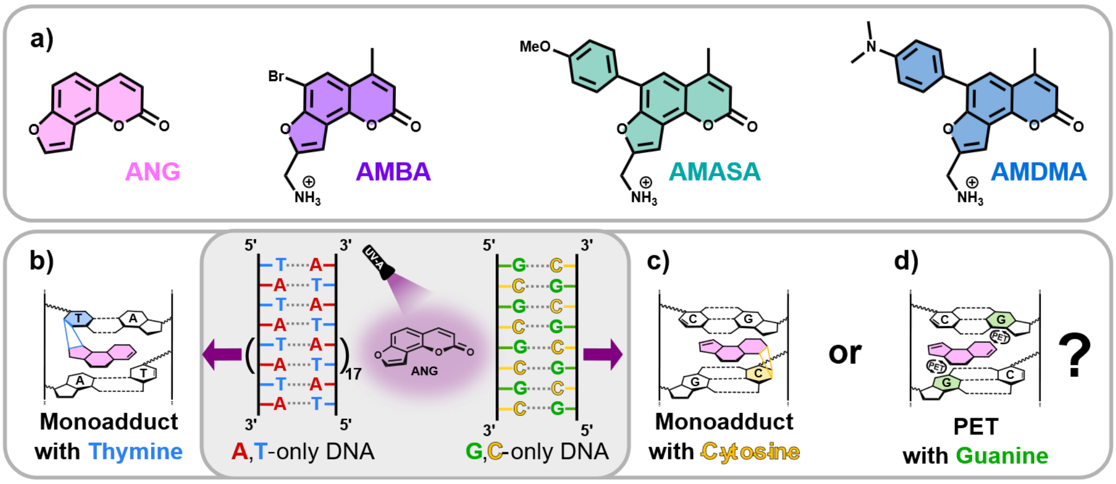

Figure 5: Investigated angelicins (a) and their potential interaction with different DNA bases (b-d).

Building on our prior work on psoralen-DNA interactions [2,4,5], we here present first spectroscopic data on angelicin (ANG) as well as novel derivatives AMBA, AMPA, and AMDMA. ANG shows limited intercalation ability and water solubility. In contrast, functionalized derivatives exhibit improved properties. Preliminary time-resolved measurements of AMBA reveal PET signatures in the presence of G (Fig. 1d), an effect not previously described for angelicins. The photophysical behavior of AMPA and AMDMA, with higher electron density and presumably suppressed PET, is currently under investigation.

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Pterin-Thymidine Adducts

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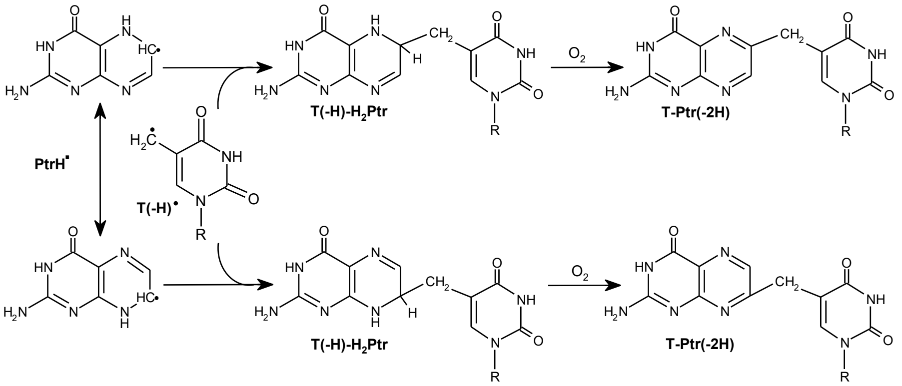
Keywords: DNA, pterin, photosensitization

Pterin (Ptr) is the model compound of aromatic pterins, which are efficient photosensitizers present in human skin, able to oxidize biomolecules upon UVA irradiation.[1] Photosensitization involves a chemical alteration of a biomolecule as a result of the initial absorption of radiation by another chemical species, the photosensitizer.[2] Under anaerobic conditions Ptr reacts with thymine (T) to form photoadducts (T-Ptr) (Scheme). In this work we present a method to prepare and purify T-Ptr adducts, using 2'-deoxythymidine 5′-monophosphate (dTMP) and single stranded oligonucleotide 5′-d(TTTTT)-3′ (dT5), and investigate their photosensitizing properties. Interestingly, Ptr moiety, when attached to T, retains its photophysical properties.[3,4] The adduct dTMP-Ptr, upon excitation, forms singlet and triplet excited states, the latter being cable of transferring energy to dissolved O_2 and generate singlet oxygen, with an efficiency similar to Ptr. In air-equilibrated solutions, both dTMP-Ptr and dT5-Ptr adducts are able to photosensitize the oxidation of tryptophan and 2′-deoxyguanosine 5′-monophosphate, two of the main targets of photosensitization in biological systems, with efficiencies close to that of free Ptr. The mechanisms involved in the oxidation of biomolecules can be either type I (electron transfer) or type II (singlet oxygen).



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Biotin-Functionalized Aloe Emodin: A Novel Photosensitizer for Receptor-Mediated Photodynamic Therapy

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Keywords: Aloe emodin, Photodynamic therapy, Biotin, Breast cancer, Photosensitizers.

Cancer continues to be one of the primary causes of death worldwide, highlighting the urgent need for more effective and selective therapeutic strategies. Among these, photodynamic therapy (PDT) has emerged as a promising technique that relies on the activation of a photosensitizer (PS) by light to produce cytotoxic effects in cancer cells.[1] Aloe emodin (AE), a naturally occurring anthraquinone, possesses multiple pharmacological properties, including antibacterial, antiviral, antimicrobial, anti-inflammatory, and antioxidant activities. In addition, AE has shown notable antitumor effects across various cancer types. Both *in vitro* and *in vivo* studies have demonstrated that AE can trigger apoptosis and inhibit tumor progression upon exposure to light.[2] Nevertheless, the clinical potential of AE as a PS in PDT is constrained by its lack of selectivity for tumor cells, emphasizing the need for targeted delivery strategies.

To improve AE's tumor selectivity and accumulation, this study explores the incorporation of biotin—a targeting agent recognized by overexpressed receptors on cancer cells—into its structure. A novel Aloe emodin-biotin analog (AE-B7) was synthesized to enhance PDT efficacy and selectivity via receptor-mediated uptake in breast cancer cells. The ester linkage was strategically designed for controlled release under physiological conditions, enabling targeted intracellular delivery. The synthesis of Aloe emodin-biotin was performed via a carbodiimide/DMAP-mediated esterification between the hydroxyl group of Aloe emodin and the carboxylic acid of commercially available biotin, a targeting agent for tumor cells. Comparative *in vitro* studies on the cytotoxicity and phototoxicity of AE and its biotin-conjugated analog were carried out using breast cancer cell lines. The biotin-conjugated derivative exhibited significantly enhanced phototoxicity under blue light irradiation. These findings support the improved efficacy of biotin-functionalized Aloe emodin in PDT, highlighting the role of biotin receptors in facilitating targeted delivery of photosensitizers.

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Contribution of 2,4-Heptadienal, an oxidation product of ω-3 polyunsaturated fatty acids, to photoreactivity of peroxidised docosahexaenoate.

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Keywords: retina, unsaturated lipids, oxidation, photoreactivity

The mammalian retina contains a high level of phospholipids esterified with polyunsaturated fatty acids (PUFA), including docosahexaenoic acid (22:6) [1], which are especially susceptible to oxidation. Highly reactive products of PUFA oxidation could irreversibly modify key cellular constituents and ultimately lead to the onset of degenerative processes [2]. It has been shown that lipids extracted from human and bovine retinas are photoreactive and under irradiation with blue light may generate reactive oxygen species [3]. Moreover, it has been demonstrated that oxidised docosahexaenoate exhibit potent photoreactivity, which may contribute to the observed phototoxicity of retinal lipofuscin [4]. In this work, we analysed photochemical properties of 2,4-heptadienal, which is a characteristic product of oxidation of ω-3 polyunsaturated fatty acids absorbing short-wavelength visible light. Photoreactivity of 2,4- heptadienal was studied by EPR-oximetry and EPR-spin trapping. Quantum yield of singlet oxygen generation by 2,4-heptadienal was determined using time-resolved phosphorescence at 1270 nm. Possible formation and presence of 2,4-heptadienal in the retinal lipid extracts was monitored using HPLC and Raman spectroscopy. Obtained data showed that upon irradiation with blue light 2,4-heptadienal generated singlet oxygen with quantum yield about 0.1, which is slightly higher than that of human retinas lipid extract, and superoxide anion. Although obtained results revealed rather moderate photoreactivity of 2,4-heptadienal, they suggested also that this species may contribute to the observed photoreactivity of oxidised retinal lipids.

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The Genesis of Porphyrin Virucidal Photosensitizers from Abiotic Processing of Glycine

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Keywords: Porphyrin; Glycine; Photosensitizer; Virucidal; Carbon Polymer Dots

EXTENDED ABSTRACT

Porphyrins are well-known photosensitizers with several applications in oncological and antimicrobial photodynamic treatments thanks to the oxidative potential of singlet oxygen ($^1O_2$). However, their clinical advancement is still hindered by the poor solubility in water and complex multi-step synthetic procedures.[1]

Our study, revisiting a typical abiotic route for oligopeptide synthesis[2], unveils for the first time glycine as an effective and single precursor for the genesis of porphyrin derivatives with a straightforward one-pot thermal procedure.

Structural, dimensional, morphological and optical characterizations highlight the generation and self-assembly of these species within a carbonized polymeric matrix in the form of water-soluble and dual blue/red emitting Carbon Polymer Dots.

The nanosystems exhibit efficient $^1O_2$ generation under visible light exposure during Indocyanine Green photodegradation tests[3] and elevated biocompatibility in Vero E6 cell line. Notably, the porphyrin-like dots also display significant photoinduced virucidal activity against Vaccinia virus, reaching a 92% of virions inactivation.

These findings propose the produced porphyrin-like dots as promising theranostic tools for further biomedical investigations, combining potential diagnostic and therapeutics functions for antimicrobial or oncological photodynamic therapy.

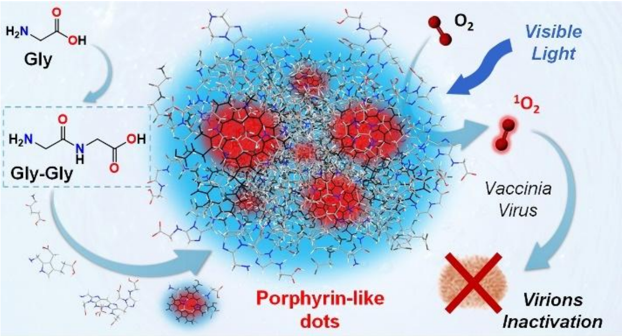

Figure 1. Schematic representation of the genesis and application of the porphyrin-like dots.

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Photodynamic inactivation of *Alicyclobacillus acidoterrestris* spores using different photosensitizers and Xenon Test Chamber Q-SUN®

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Keywords: APDT; Spoilage; Fruit juice industry; Mathematical modelling

The presence of *Alicyclobacillus acidoterrestris* spores may cause fruit juice spoilage mainly due to guaiacol production since pasteurization is not able to inactivate spores during the processing stage in fruit juice industry. The present study aims to evaluate the efficacy of antimicrobial photodynamic treatment (APDT) using new methylene blue N (NMBN), methylene blue (MB), curcumin (CURC), 8-methoxypsoralen (8-MOP), chlorin e6 (Ce6), and purpurin-18 (P18) as photosensitizers (PS) in the presence and absence of potassium iodide (KI) as a potentiator agent against *A. acidoterrestris* spores. Stock solutions of the PS were prepared using sterile deionized water (NMBN and MB) or DMSO (CURC, 8-MOP, Ce6, and P18). Light exposure was carried out using the Xenon Test Chamber Q-SUN® (Model XE-3-HC) to simulated full-spectrum solar radiation. The spore suspension of *A. acidoterrestris* (DSM 2498) was prepared in fifty Roux bottles containing Yeast-Starch-Glucose Agar (YSG-A), pH 3.7±0.1, and supplemented with 10 ppm manganese sulfate. The inoculated Roux bottles were incubated at 45 °C for 30 days. The process of spore formation was continuously monitored by staining with malachite green and observing in optical microscope (100 ×). The final concentration of the spore suspension was 7.25×10^8 spores/mL. The best conditions for APDT against *A. acidoterrestris* spores were determined by minimal inhibitory concentration (MIC) assay. Briefly, 50 µL of spore suspension (3.00×10^3 spores/mL), 50 µL of a PS solution (3× concentrated) and 50 µL of KI (30 mM) or sterile deionized water were added to each well of a 96-well plate. Plates were kept in the dark for 30 min before light exposure for 30, 60, 90 and 120 min. Then, 150 µL of 2× concentrated YSG broth pH 3.7±0.1 were added to each well and immediately incubated at 45 °C for 48 h in the dark. Light and dark controls were included to evaluate the isolated effects of full-spectrum solar radiation and PS, respectively. Survival curves were generated by plotting logarithmic population counts (Log_{10} spores/mL) *versus* fluence (J/cm^2). The inactivation data were analyzed using the GInaFiT Excel® add-in for mathematical modeling, GraphPad Prism 6® for graphical representation, and Minitab 16® for statistical analysis (ANOVA with Tukey's post-hoc test, $p < 0.05$). The MIC assay results demonstrated that APDT with NMBN, MB and P-18 achieved a reduction of *A. acidoterrestris* spore viability only in the presence of KI (10 mM). On the contrary, APDT with CURC, Ce6 and 8-MOP reduced *A. acidoterrestris* spores viability both in the presence and absence of KI (10 mM). Ongoing experiments are using intervals of concentrations of 50 – 150 µM of NMBN and MB, as well as 25 – 100 µM of P-18, in the presence of KI (10 mM). Additionally, tests are being conducted with 10 – 50 µM CURC, 25 – 100 µM Ce6, and 8-MOP, both with and without KI (10 mM), to determine the kinetic parameters of *A. acidoterrestris* spore inactivation. Results obtained in this study will contribute to future APDT developments for field and industry applications.

Phototaming of Bacterial Bioelectricity

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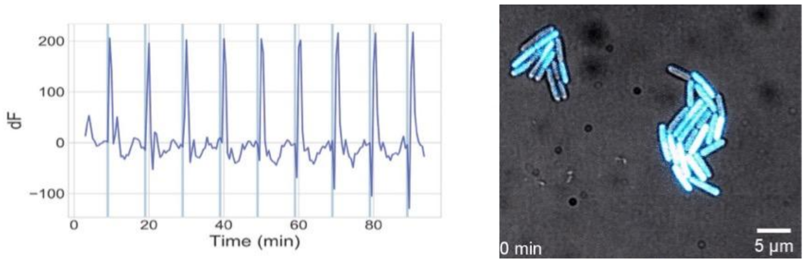
The goal of engineering living matter is to modify biological attributes to leverage the unique capabilities of living organisms. One prevalent method involves rendering living matter responsive to specific stimuli through either synthetic biology techniques or functional materials, aiming to modulate the electrophysiology and activity of cells and organisms. This method applies to bacteria as well, even though the connections between their electrophysiology, bioelectricity, bioenergetics, and behavior have only recently started to be elucidated. Recent studies have revealed that bacterial membrane potential is a dynamic, rather than static, parameter and plays a significant bioelectric signaling role. Such a communication paradigm governs their metabolism, behavior, and functions within microbial communities. Given that membrane potential dynamics mediate this language, manipulating this parameter represents a promising and intriguing strategy for bacterial engineering.

Here, I show that precise optical modulation of bacterial membrane potential can be achieved through a materials-based approach.[1] Specifically, we found that the isomerization reaction at the membrane location induces either hyperpolarisation or depolarisation of the potential depending on the excited state deactivation pathways, within a bio-mimetic mechanism reproducing the initial fate of retinal. This can trigger neuron-like bioelectric signalling and can highlight the role of previously uncharacterized ion channels in bacteria electrophysiology. Finally, I also show perspectives on the light-modulation of antibiotic uptake, as well as on the photocontrol of bacterial motion and assembly behavior in consortia and multispecies ecosystems.



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Revealing the role of membrane lipidic environment in photo-regulation: antenna protein CP29 and its mutants inside nanodiscs

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Keywords: Photosynthesis; Antenna complex; Energy transfer; Optical spectroscopy

Light harvesting complexes (LHCs) exhibit a dual nature; under low light conditions, they maximize the solar photon collection for photochemistry, whereas under excess light conditions they dissipate the excess absorbed energy as heat, to avoid photodamage of the delicate inner components of photosynthesis. Under stress, photosystems utilize different mechanisms to regulate their functions, which are collectively referred to as non-photochemical quenching (NPQ). The molecular mechanisms of NPQ are still debated as it is a very complex process which requires multiple interactions between chromophores, either chlorophylls, carotenoids or both. Here, we explore the quenching mechanisms of the minor antenna complex CP29 by the combination of site-directed mutagenesis with picosecond time-resolved fluorescence and femtosecond transient absorption (TA) spectroscopies. The measurements are performed on CP29 and mutants inserted in nanodiscs to mimic the native membrane environment. Our study provides experimental evidence on involvement of multiple Chl–Car interactions both in L1 and L2 quenching sites of CP29 in the photoprotection mechanism. In case of A_2 mutant (selective removal of Chl a612), Chl fluorescence lifetime becomes longer compared to CP29 wild type (WT). On the other hand, the A_5 mutant (site Chl a603, mutation H111N) which increases excitonic interactions between Chl a603 (A5) and Chl a609 (B5), exhibits faster Chl fluorescence lifetime compared to WT. Global analysis of the TA data suggests the presence of energy transfer process from Chl a Q_y to a Car dark state S* in both the A_2 and A_5 mutants. Our study provides experimental evidence that multiple Chl–Xant interactions are involved in the quenching activity of CP29.

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Conjugated polymer nanoparticles for biophotonic applications: biohybrid photo-sensitive interfaces for living cells

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Keywords: biophotonics, conjugated polymers, biohybrid systems

Biophotonic technologies hold immense promise for applications spanning from theranostics to plant engineering [1], [2].

We explore conjugated polymer nanoparticles (CP-NPs), specifically poly(3-hexylthiophene) (P3HT), [6,6]-phenyl C61-butyric acid methyl ester (PCBM), and their blend (P3HT:PCBM), as functional nano-materials for the development of photo-active biohybrid systems [3], [4]. The CP-NPs were synthesized via nanoprecipitation, resulting in stable colloidal dispersions with average diameters of ~180 nm. Optical and electronic characterization revealed strong absorption in the 350–600 nm range and emission in the red to near-infrared region (650–900 nm), aligning well with biological spectral windows. When exposed to light in phosphate-buffered saline solution (PBS), the CP-NPs generated photocurrents in the range of 0.18–7.0 nA cm^{-2}, evidencing efficient conversion of light to electrical signals in aqueous environments. Simulations of their interaction with biological media under illumination revealed robust carrier generation, up to 3×10^{20} nA cm^{-3} for P3HT-NPs, supporting their potential for light-triggered sensing or stimulation in physiological environments.

Extending this approach to plant photobiology [5], we integrated P3HT-NPs into *Arabidopsis thaliana* to create a biohybrid plant. The P3HT-NPs' absorption spectrum shows complementarity with the chlorophyll absorption bands, thereby augmenting the light-harvesting capacity of the plant. Notably, P3HT-NPs trated plants showed an increase of up to 45% in root length and ~17% in total biomass production compared to untreated controls. These enhancements point to improved CO_2 assimilation.

In conclusion, CP-NPs can serve as external antennae and photoactive interfaces within plants. This biohybrid strategy highlights a photobiologically relevant synergy between nanomaterials and plants, pointing to innovative applications in sustainable agriculture and renewable energy, from more efficient CO_2 capture via increased biomass production to the development of engineered light-responsive biohybrid living systems.

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Fluorescence proteins: a light management mechanism in Corals

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Keywords: Fluorescence proteins, corals, time-resolved fluorescence spectroscopy, energy transfer

Corals are marine invertebrates and the primary builders of reefs. The basic unit of a coral is called a *polyp*, characterized by one or more rings of tentacles surrounding a central mouth. The internal cavity, often referred to as the "stomach skin," is lined by a tissue called the gastroderm, which hosts golden-brown symbiotic algae. This symbiosis makes corals polytrophic, combining both photosynthetic capabilities and a digestive system. In addition to the pigments produced by the algae, such as carotenoids and chlorophylls, corals can express fluorescent proteins (FPs), which are responsible for the striking colors observed in many species. In this research, we investigated the role of FPs in light management mechanisms, focusing on a *Favia sp.* coral with a phenotype we refer to as "Favia Green." Specifically, we studied energy transfer mechanisms involving the coral's fluorescent proteins. Pigment extraction revealed a peak corresponding to green fluorescent protein (GFP, emission between 500–570 nm) and another corresponding to cyan fluorescent protein (CFP, emission below 500 nm). Hyperspectral imaging allowed us to map the distribution of these pigments within the coral colony, identifying two main regions: the mouth and the surrounding tissue. Fluorescence microscopy showed more densely packed granules in the tissue, and optical coherence tomography revealed this region also exhibits higher light scattering compared to the mouth. Additionally, we found that the tissue region reaches photosystem saturation at higher light intensities than the mouth. This difference may result from a combination of energy transfer phenomena and enhanced scattering in the tissue. Notably, the spectral overlap between CFP emission and chlorophyll absorption suggests that photons emitted by CFP can be transferred to GFP and subsequently re-emitted at wavelengths where chlorophyll absorption is low. We also observed fluorescence decays faster in the tissue than in the mouth for the CFP using time resolved photoluminescence setup and supporting the energy transfer phenomena. Regarding scattering, the FP granules in the tissue scatter more photons than those in the mouth region. This results in fewer photons reaching the photosystems located beneath the tissue. The combined effects of energy transfer and scattering may represent a light management strategy used by the coral to protect its symbiotic algae from excess light.

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Optical Characterization of 3D Light-Driven Bio-Hybrid Actuators

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Bio-hybrid actuators represent a promising field in robotics. The advantage of a 3-dimensional device is that it can better mimic a human muscle and achieve high performance, adaptability, and complexity of movement, matching the standards required in the robotics field [1].

Muscle-based biohybrid systems are typically triggered electrically using electrodes. However, this approach has limitations, such as low spatial resolution and selectivity, potential tissue damage from inflammatory responses, and cumbersome wiring [2]. To address these issues, scientists are exploring light as an alternative trigger. Light stimulation offers high spatial and temporal resolution, low invasiveness, and can be controlled remotely. Methods like infrared radiation, semiconductor interfaces, and optogenetics have been successfully used to induce light-cell sensitivity. Another promising approach is the use of photoactive molecules. Recently, it has been demonstrated that an azobenzene molecule called Ziapin2 can modulate membrane capacitance and trigger muscle cells' contraction upon light stimulation in 2D systems [3], due to its light-triggered isomeratation process in the cell's membrane. Starting from this promising result, we infer that this stimulation approach may be used to induce the deformation and contraction of a 3D bio-hybrid actuator.

Here we describe an example of a 3D bio-hybrid structure treated with Ziapin2 that is capable of contracting upon light stimulation. We studied and characterized the optical properties first of a 3D-printed hydrogel based on gelatin methacrylate. Subsequently, we encapsulated the skeletal cells inside the hydrogel to study Ziapin2 uptake and diffusion inside the cells and the hydrogel. Our focus was on optimizing the uptake of Ziapin2 to enhance its interaction with light, thereby achieving the most effective contractions. This study represents a significant step towards developing light-sensitive bio-hybrid actuators and a fundamental study of their optical properties.

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Engineering of Light Sensitivity in Chlamydomonas reinhardtii

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Keywords: Chlamydomonas reinhardtii; Phototaxis; Biohybrid microrobots; Light-responsive materials; Azobenzene photoswitches; Fluorescence Lifetime Imaging (FLIM); Synthetic phototransduction ;

The evolutionary ability of certain microorganisms, such as bacteria and microalgae, to convert light energy into active motion makes them ideal candidates for the development of biohybrid microrobots capable of navigating highly viscous and confined environments, ranging from biological tissues to complex microfluidic architectures. In this context, Chlamydomonas reinhardtii (wild type, strain 124 mt–) stands out as a privileged model due to its natural phototaxis[1], intrinsic chlorophyll fluorescence, and compatibility with non-genetic engineering strategies.

This study investigates phototaxis as both an activation and control mechanism, intending to harness live cells as functional units within light-responsive, bioinspired microsystems for applications in targeted delivery, guided transport, and environmental sensing[2]. To this end, we designed and built a custom fluorescence microscopy platform equipped for dynamic phototactic stimulation, enabling precise control over light parameters (wavelength and intensity) and allowing high-resolution analysis of motility metrics such as speed, trajectory, displacement, and directionality.

To further enhance and modulate light responsiveness, we are engineering Chlamydomonas using exogenous photoactive molecules, specifically Ziapin2[3], an azobenzene-based compound capable of light-induced membrane potential modulation. Spectroscopic characterisation in live-cell suspensions (Kautsky kinetics, absorption spectra, etc.) revealed preferential localisation of the molecule in lipophilic environments and suggested a functional effect on membrane polarisation. We also identified working concentrations compatible with cell viability, currently employed in phototaxis assays.

Finally, we are monitoring real-time photosynthetic dynamics during phototactic behaviour using a custom Fluorescence Lifetime Imaging Microscopy (FLIM) [4] system integrated with standard imaging. This tool enables subcellular resolution mapping of chlorophyll fluorescence lifetimes, allowing us to correlate photophysical responses with light-induced motility patterns.

Preliminary results demonstrate a robust directional response to specific wavelengths and suggest that molecular photoactuation can alter both the fluorescence signature and phototactic behaviour of the cells. These findings lay the groundwork for light-guided algal microswimmers and contribute to the broader vision of autonomous, bio-compatible microrobots for biomedical and environmental applications.

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B. subtilis membrane potential photomodulation through newly-synthesized azobenzenes.

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Keywords: Optogenetics, Electrophysiology, Bacteria, Azobenzenes

EXTENDED ABSTRACT

Over the past fifteen years, research has increasingly focused on bacteria as dynamic living organisms, beyond their role as potential pathogens. Bacteria exhibit "neuron-like" behaviour, relying on electrical signalling for communication[1]. A key enabler of this behaviour is the membrane potential, regulated by ion flux across the cell membrane, which influences motility, pH balance, and nutrient uptake. To modulate bacterial membrane potential, photosensitive azobenzenes offer promising solutions[2]. These organic molecules undergo light-triggered trans-cis isomerization, allowing precise, real-time control of ion flux and electrochemical responses[3].

In this study, we present the electrophysiological characterisation, obtained through the use of fluorescence-based microscopy and the crucial contribution of voltage-sensitive Nernstian dyes, of *B. subtilis* in presence of some newly synthesised azobenzene-based molecules. Particularly, we will focus on the possibility to modulate the membrane potential through various light at various wavelengths and on the role of specific ion channels in potential modulation and in the general homeostasis. By combining our molecules with specific engineered *B. subtilis* mutans strains we will not only be able to show the possibility to precisely modify membrane potential through time, but we will also highlight the molecular mechanisms of adaptation to cope with this perturbation.

Our findings offer valuable insights on the still partially unknown functioning of bacteria and open interesting scenarios towards the possible effect of real-time photomodulation of membrane potential including the study of their response and resistance against antibiotics.

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NOVEL PHOTOACTUATORS FOR BACTERIAL MODULATION: DESIGN, SYNTHESIS AND CHARACHTERIZATION

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Keywords: Photochromism, Bacteria, Membrane potential

In the field of cellular stimulation, light offers a high spatiotemporal precision control with minimum invasiveness, and it allows for remote control of biological functions when combined with photoswitches. In this work, different photochromic systems have been designed, synthesized and characterized to target bacteria and to photomodulate their membrane potential. In contrast with eukaryotic cells, important processes regarding bacteria functioning, such as ATP generation and signalling, occur at plasma membrane level. In particular, bacteria membrane potential plays a key role in signalling and adaptation to antibiotics. Here, we show how the functionalization of azobenzenes allows for tuning light absorption and quantum efficiency, which play a fundamental role in light penetration for clinical applications. Specifically, donor-acceptor strategy has been exploited to strongly red-shift absorption, and the use of electron-poor heterocycles turns out to be effective both in red shifting the absorption band and in imparting an amphiphilic character to the photochrome. The latter characteristic is essential to drive a spontaneous partitioning of the photoswitch into the bilayer. Preliminary experiments have been carried out on bacteria (*B.Subtilis* and *E.Coli*) to investigate localization and toxicity, further assays will be performed to evaluate photomodulation of membrane potential. Conferring light sensitivity to bacteria allows for the creation of novel biohybrid materials which can offer new solutions in the fields of drug delivery and minimization of antibiotics resistance. [1], [2], [3].

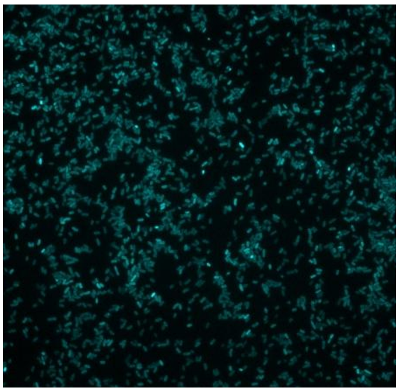

Figure 6 *B.Subtilis* treated with novel photoswitches

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Spatiotemporal Control of Bacterial Adhesion via Light-Responsive Modulators

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Keywords: AMR; Biofilm; Photopharmacology; Photoswitchable

Antimicrobial resistance (AMR) poses a growing global health threat, particularly in the context of hospital-acquired infections. One of the major contributors to the persistence and spread of AMR is biofilm formation, which creates a protective microenvironment that enhances microbial survival and facilitates horizontal gene transfer [1]. Central to biofilm development are bacterial adhesion proteins, which mediate crucial interactions between microbial cells and surfaces, thereby supporting the proliferation of highly resistant microbial communities. Owing to their pivotal role in biofilm formation, adhesion proteins have emerged as attractive targets for the development of novel anti-AMR strategies [2,3].

Among these targets, the virulence factor LecB-lectin, located on the outer membrane of *Pseudomonas aeruginosa*, has received particular attention. LecB not only contributes significantly to the pathogen’s virulence and persistence in clinical settings, but also demonstrates strong carbohydrate-binding activity, interacting with both the bacterial outer membrane and exopolysaccharides within the biofilm matrix.

This project aims to identify and develop innovative photoswitchable ligands capable of binding and modulating LecB activity. Utilizing light-responsive mechanisms, these ligands are designed to offer a novel therapeutic approach with high spatiotemporal control and minimal invasiveness, potentially overcoming limitations associated with traditional antimicrobial treatments. Beginning with the identification of an initial photoswitchable *hit* compound, which provides proof of concept for this therapeutic strategy targeting bacterial adhesion proteins, right after we started a computationally guided *hit expansion* program. This approach sought to discover new photoswitchable binders with enhanced affinity in the *cis*-enriched form for evaluating light-dependent antibiofilm properties of the compounds. The design rationale, synthetic pathways, photochemical characterization, and preliminary biological assessment of these compounds will be presented and discussed.

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Investigating The Mechanism of Cardiac Cell Excitability Modulation by a Membrane-Targeted Photoswitch

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Keywords: non-genetic light stimulation; photoactuators; cardiac cellular modulation.

The use of light to control cellular activity presents a promising approach in cardiac research due to its precise stimulus localization and minimal invasiveness [1]. Ziapin2, a membrane-targeted azobenzene compound, has already been identified as an effective tool for light-driven modulation of excitation-contraction coupling (ECC) in hiPSC-derived cardiomyocytes [2,3]. Its mechanical photomodulation of membrane thickness leads to changes in membrane capacitance (Cm), which are linked to membrane potential alterations that trigger action potential (AP) generation. Despite a robust physical interpretation, a detailed biophysical explanation of this process remains under investigation. To further explore this, we tested Ziapin2 in a more mature model: adult mouse ventricular cardiomyocytes (V-CMs). Using standard electrophysiological techniques and enhanced computational models, we delved deeper into the biophysical mechanisms. Our in vitro results demonstrate that Ziapin2 can photomodulate ECC in mature V-CMs without affecting the main transporters and receptors located within the sarcolemma. Furthermore, we experimentally established the connection between Ziapin2-induced membrane thickness modulation and light induced AP firing by showcasing the pivotal role of stretch-activated ion channels (SACs) through pharmacological blockade. Our experimental findings were successfully supported by mathematical simulations, incorporating Cm changes and SACs activation due to membrane tension caused by Ziapin2-induced thickness modulation [4]. Together, these results enhance our understanding of the biophysical processes involved, shedding light on the mechanism of action of Ziapin2 as a novel, precise, and non-invasive tool for controlling cardiac electrical activity.

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UV radiation and climate effects through the lens of the Environmental Effects Assessment Panel
Chair: Janet Bornman

How stratospheric ozone, air-pollution, and climate will shape surface UV radiation in the 21st century?

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Keywords: UV radiation, UV projections

The interactive effects of stratospheric ozone and climate change on solar UV radiation in the 21st century involve a complex interplay of anthropogenic influences and natural processes. Understanding these interactions is essential for developing effective strategies to mitigate the adverse impacts of ozone depletion and global warming on ecosystems, human health, and the environment. The levels of UV radiation are primarily determined by atmospheric processes, including attenuation by ozone, clouds, and aerosols, as well as enhancement by the reflectivity of Earth's surface.

The onset of ozone depletion in the 1980s led to increased levels of solar UV-B radiation at the surface—particularly in the high latitudes of the Southern Hemisphere—a trend that continues despite signs of ozone recovery. However, the projected "super-recovery" of total ozone toward the end of the 21st century, potentially exceeding pre-depletion levels, is expected to result in a decrease in UV-B radiation. These variations in UV-B exposure may have already affected—and are likely to continue affecting—human health and ecosystems in diverse ways.

Moreover, climate change driven by increasing greenhouse gas concentrations influences clouds, surface reflectivity, and, to some extent, aerosols. These factors further complicate our ability to project future trends and variability in solar UV radiation, as well as the associated environmental effects. Due to regional variability in these influencing factors, different areas of the world are expected to experience distinct impacts.

Projections of ozone, cloudiness, surface reflectivity, and aerosols for the 21st century, as predicted by state-of-the-art Earth system models, are used in radiative transfer simulations to assess the global variability and changes in surface UV radiation [1, 2]. These projected changes, analyzed under different socioeconomic pathways, are discussed in terms of their implications for human health and ecosystems.

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Integrated global assessment 0f the interactive effects of UV radiation, climate and stratospheric ozone depletion on polar and alpine ecosystems, photodegradation of plant material and of pesticides.

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Interactions between climate change and ozone depletion continue to alter the environment and to affect ultraviolet-B radiation. UV-B radiation has significant impacts on marine and terrestrial ecosystems, particularly in high-latitudes regions. An extended period of Antarctic ozone depletion during 2020-2023 has led to prolonged exposure to UV-B radiation in southern high latitudes. This affects organisms during spring and summer often coinciding with their reproductive period and this effect is exacerbated by record low seas ice and early snowmelt. Antarctic and Patagonian ecosystems are most affected, with species at the base of the food chain potentially incurring energetic costs from up-regulation of UV-protective mechanisms. Anthropogenic climate change is also driving global glacier retreat, which reveals new ground for colonisation in alpine and polar environments. The colonisation of these ecosystems may be altered if early successional UV-adapted organisms struggle to cope with rising temperatures. Globally, UV radiation plays a crucial role in photodegradation processes affecting the rate of carbon cycling. Litter decomposition is accelerated by the direct photochemical mineralisation of lignin and by enhanced microbial activity (photofacilitation), with climate and land-use changes further modulating these effects. Additionally, UV-B radiation affects the persistence and toxicity of pesticides, impacting environmental safety and food security. Understanding these interactions is essential for predicting the future consequences of ozone recovery, climate change and land-use shifts for both ecosystems and biogeochemical cycles.

Potential impacts of solar radiation modification (SRM) on ecosystems

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Rising global temperatures pose significant risks to ecosystems, biodiversity and global food security. Recent comprehensive assessments suggest that large-scale mitigation efforts to limit warming are falling short, and all feasible future climate projections, including those that represent optimistic emissions reductions, exceed the Paris Agreement's <1.5°C or 2º warming targets during this century. While avoiding further CO_2 emissions remains the main and most effective way to avoid further destabilizations to the environment, a growing body of research has now aimed to explore various proposed climate interventions. Through deliberate manipulation of the environment at large scales, one such intervention strategy could help to ameliorate anthropogenic global warming by decreasing surface temperatures through increased sunlight reflection (solar radiation modification, SRM). Yet, there is a high level of uncertainty in how ecosystems will respond to SRM directly as well as how global scale climate intervention may impact ecosystems' responses to climate change. SRM may have the potential to reduce warming-driven impacts but could also substantially alter food systems and ecosystem function. This research provides an overview of SRM as related to ecosystem impacts, emphasizing knowledge gaps and the need for improved predictive models.

PFAS or non-PFAS ? The photochemistry of new(er) CFC replacements and implications for climate change

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Confusion about definitions of per- and polyfluorinated alkyl substances (PFAS) cloud technical, regulatory, and public discussions of the compounds, and hence their relationship to the Montreal Protocol. Nevertheless, concerns have been raised that certain Montreal Protocol controlled substances and their alternatives and breakdown products, are per- and polyfluorinated alkyl substances (PFAS), and/or degrade to give PFAS, some of which are persistent, toxic, and threaten global ecosystems and human health.

Trifluoroacetic acid ($CF_3C(O)OH$, TFA) is considered a PFAS under some definitions and has been a focus for discussion as a persistent degradation product from compounds under the purview of the Montreal Protocol. Some CFC replacement compounds have short, extended perfluorinated carbon-chains (C_xF_{2x-1}-units where x = 2,3), which means that short chain-length PFCAs (perfluoroalkyl carboxylic acids), in addition to TFA, are also likely to be found in the environment.

Trifluoro methane, CF_3H (HFC-23), is a long-lived greenhouse gas (GHG) with a 100-year global warming potential (GWP_{100}) of 14,600. HFC-23 is persistent, but not considered a PFAS under any the definitions of PFAS. Historically, byproduct emissions from HCFC-22 ($CHClF_2$) production have been the largest contributor to the atmospheric abundance of CF_3H. Emission reductions, supported by the UNFCC's Clean Development Mechanism (CDM), has led to a substantial decrease in CF_3H emissions, as reported and verified through the CDM projects. However, a significant and persistent gap has emerged over the last decade between estimated (from atmospheric measurements) and reported emissions of CF_3H.

Trifluoro acetaldehyde, CF_3CHO, which is a product of atmospheric degradation of some halogenated olefins (HFOs/HCFOs), hydrochlorofluorocarbons (HCFCs) and hydrofluorocarbons (HFCs), can undergo reactions in the atmosphere to give TFA in a few percent yield. However, trifluoro acetaldehyde also undergoes photolysis leading to trifluoro methane under certain atmospheric conditions. Ozonolysis of some commercially relevant HFOs have recently been shown to be a source of CF_3H. This could effectively present a significant additional secondary contribution to the radiative forcing of climate from the parent CFC alternatives.

Over the past two years we have conducted chamber experiments and modeling studies investigating the photolysis kinetics and products of CF_3CHO under conditions relevant to the troposphere and the stratosphere. We have also studied the ozonolysis of $CF_3CH{=}CHCl$, a widely used hydrochlorofluoroolefin (HCFO-1233zd).

These findings are discussed in the context of the atmospheric fate of CF_3CHO, the contribution of HFOs to the radiative forcing of climate change, to the environmental deposition of TFA and the atmospheric abundance of CF_3H.

UV radiation and climate effects on fiber material degradation: Challenges and sustainable design

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Keywords: Fiber materials; Nanomaterials; Natural sources; Degradation

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.

Clothing fabrics and other fiber materials are usually exposed to the outdoors, where UV radiation from sunlight, as well as climatic factors such as temperature, humidity, and atmospheric pollutants, can adversely affect their lifespan. These factors can induce material degradation, resulting in the release of small molecules within its structure that cause health hazards and environmental pollution. Therefore, achieving a balance between material performance and sustainability is imperative. Researchers modify fiber materials to develop advanced functional materials with specific UV-resistant properties, such as improved durability and stability. In this report, we will first review various modification methods for UV resistance and thermal radiation protection in fiber materials, including nanoparticles, organic modification, and surface structures. Secondly, we will also summarize the research progress on application of plant extracts from natural and sustainable sources such as tannins, tea polyphenols, and lignans for UV resistance in fibers and fabrics. Finally, we will briefly introduce how we design nanomaterials for the efficient degradation of toxic compounds and plastic in consideration the role of UV-radiation.

Effects of solar visible blue light on human skin aging and pigmentation - current status and options for protection and treatment

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Keywords: sun protection, high-energy visible light, oxidative stress, pigmentation

EXTENDED ABSTRACT

Currently, skin photo-protection primarily focuses on the UV-portion of the solar spectrum since the high energy of UV-radiation induces skin damage after a short time of exposure. Consequently, sunscreen products contain effective UV filters to provide high UVA and UVB protection. However, there is accumulating evidence that also high energy visible light (HEVIS, 400-500 nm) significantly affects skin physiology. Since conventional UV filters do not protect against visible light, other means of photo-protection are needed to counteract HEVIS-induced reactive oxygen species (ROS) production.

The symposium's objective is to present new data on the effect of the visible light part of the solar spectrum, especially the blue part, on the physiology of human skin. Damage responses and means to protect the skin will be discussed in various presentations.

Analytical imaging and functional analysis of reactive lipids – connecting photodamage and senescence

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Keywords: photodamage, senescence, reactive oxidized lipids

Exposure of biological material to specific wavelenght bands of radiation can result in oxidative modification of intra- and extracellular macromolecules. This can happen through the non-enzymatic generation of reactive oxidized lipids. Such lipids also accumulate in- and are secreted by senescent cells. Among the lipids increased by exposure to UVA and -at a lesser extent also by other wavelenght light, but also in prematurely senescent dermal fibroblasts were lipid hydroperoxides but also the aldehydophospholipids palmitoyl-oxovaleroyl-phosphatidylcholine (POVPC) and palmitoyl-oxo-nonanoyl-phosphatidylcholine (PONPC). Using HPLC-Mass Spectrometry and Maldi MS Imaging we could identify and localize such reactive aldehydolipids in skin, 3D skin equivalent models and cell free dermal equivalent models and correlate their appearance to presence of photostressed or senescent cells and reconsituted multilammelar vesicles.

We also investigated the lipid-induced collagen modifications and their effects on skin cells to assess long-term consequences of lipids from photostressed or senescent cells to the ECM and microenvironment. Using mass spectrometry and biochemical methods, we identified high- and low molecular weight modifications to collagen type I, II and IV. HNE-modified collagen reduced proliferation and induced stress responses in fibroblasts, including upregulation of HO1 and HSPA1A, while OxPL-modified collagen triggered inflammation. Both modifications affected matrix remodeling genes, they increased MMP1 and MMP3 expression while reducing collagen type I and III expression. Both HNE and OxPL modified collagen led to elevated levels of cellular reactive oxygen species (ROS) and further lipid. In addition, macrophages cultured on modified collagen displayed altered cytokine profiles and TLR4 signaling impairment. When lipid modified collagen was used to generate organotypic skin equivalents led to disturbed differentiation of epidermal keratinocytes and increased markers of cellular senescence like loss of perinuclear LaminB1, higher γH2AX and p16 levels.

Taken together, our data suggest that oxidized lipid modifications to collagen contribute to an aging phenotype in the skin. The finding that also adducted lipids interfere with PRR signaling could suggest that modified matrix reduces the immune surveillance and ability of phagocytes to recognize oxidation/senescence associated patterns and thereby may contribute to immune evasion of entities which carry oxidized epitopes, including damaged and senescent cells themselves.

Redefining sun protection – Protection strategies to defend solar induced damages beyond the UV spectrum

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Keywords: Visible Light, Photoprotection, Hyperpigmentation, Oxidative Stress

The current state-of-the art sunscreens are primarily protecting the skin against high energy UV irradiation. These products typically contain UV-filters to provide high UVA and UVB protection. However, UV irradiation marks only a small part of the solar spectrum and there is accumulating evidence that also light of longer wavelengths (> 400 nm) causes significant physiological changes of the skin. Besides the induction of reactive oxygen species (ROS), also darkening of hyperpigmented spots has been observed. In contrast, there is no convincing evidence that infrared irradiation causes similar effects. Since conventional UV filters are not capable to protect the skin from sun light above 400 nm, a special focus on pigmented or tinted formulations arose. However, these are not broadly accepted by the consumer. Thus, novel photoprotective strategies are needed to counteract the physiological changes affected by visible light (VIS).

Here we showcase the chemical molecules Licochalcone A and Thiamidol as potent protective actives against VIS. Licochalcone A is a NRF2 inducer and stimulates the intrinsic antioxidative machinery of the cells. Thus, cell cultures pre-treated with Licochalcone A significantly reduced VIS-induced ROS. Surprisingly, classical radical scavenger like Vitamin C and its derivatives ascorbyl-palmitate and ascorbyl-phosphate, which do not induce Nrf2, did not significantly reduce VIS-induced ROS. Thiamidol is a potent inhibitor of human tyrosinase and counteracts hyperpigmentation. Consequently, VIS irradiation induced melanin production in melanocytes is significantly reduced by Thiamidol treatment. This also enables the reduction of UV protected but hyperpigmented areas which cannot be constantly covered from the sunlight.

Overall, these results demonstrate novel intervention strategies in underrepresented areas of sun protection apart from classical UV protection.

Cyclic AMP Response Element-Binding Protein (CREB): A Novel Early Biomarker for Predicting the Efficacy of Sun Protective Agents in Preventing Skin Carcinogenesis

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Keywords: Photocarcinogenesis; Photoprotection; Vitamin D

Australia and New Zealand have the highest skin cancer rates globally despite ongoing sun safety campaigns. This highlights the urgent need for improved photoprotective measures. We have previously demonstrated that active vitamin D compound, 1,25-dihydroxyvitamin D_3 (1,25D), and structurally related compounds including 20-hydroxyvitamin D (20D), 1,25-dihydroxylumisterol (JN) and tetrahydrocurcumin (THC) are novel photoprotective agents. Protection against acute markers of ultraviolet radiation (UVR)-induced damage such as DNA damage and immunosuppression is often used to predict an agent's ability to protect against chronic UVR damage in a 40-week murine photocarcinogenesis protocol [1]. However, several photoprotective agents including 20D and QW have proven this correlation is not always reliable [2]. Phosphorylated cyclic AMP-regulatory element-binding protein (pCREB) is a transcription factor that is overexpressed in skin cancer and may serve as a predictive biomarker in predicting the efficacy of photoprotection by an agent. We have shown that UVR increases pCREB levels in melanocytes and keratinocytes [3]. 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 increases 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. Ongoing studies in keratinocytes show a similar trend to previous published work by De Silva and colleagues. Additionally, due to pCREB being a potential predictive biomarker for skin cancer, our novel studies using an inhibitor are being conducted to highlight the importance of this biomarker in driving photocarcinogenesis. Our results suggest that pCREB may potentially be a predictor of photocarcinogenesis and could play a role in streamling the process of identifying suitable photoprotective agents for a 40-week photocarcinogenesis model. Also, pCREB inhibition may prove to be a therapeutic approach to melanoma.

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Assessing Quality of Life in Patients with Photodermatoses: An Examination of the relationship between Dermatology Life Quality Index (DLQI), Hospital Anxiety and Depression Scale (HADS), and Patient-Reported Impact of Dermatological Disease (PRIDD) Scores and Photodiagnostic Investigation Results.

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Keywords: Photodermatoses, Quality of Life, Photodiagnostic Testing

Introduction: Photodermatoses are a heterogeneous group of skin disorders characterised by abnormal cutaneous responses to ultraviolet (UV) or visible light exposure. These conditions present unique diagnostic and management challenges and are associated with a significant burden on patients' quality of life (QOL). Rates of anxiety and depression in this patient group are reported to be approximately twice those observed in the general UK population[1]. Despite this, there are currently no validated, disease-specific QOL tools designed to assess the psychosocial and functional impact of photosensitivity. Consequently, a combination of generic and dermatology-specific tools—including the modified 12-month Dermatology Life Quality Index (DLQI), the Hospital Anxiety and Depression Scale (HADS), and more recently, the Patient-Reported Impact of Dermatological Diseases (PRIDD)—are routinely employed in our tertiary photobiology service to assess this impact. To establish objective measures of photosensitivity, patients undergo comprehensive photodiagnostic testing. The gold standard investigation, monochromator phototesting, is performed using a broadband xenon arc lamp in conjunction with a diffraction-grating monochromator, allowing precise wavelength selection. Additionally, larger-area iterative broadband ultraviolet A (UVA) provocation testing and narrowband UVB phototesting are used to enhance detection sensitivity.

Objective: This study aimed to evaluate the relationship between the impact of photosensitivity on patient-reported QOL, as measured by the modified DLQI, HADS, and PRIDD, and objective photodiagnostic outcomes, including monochromator phototesting, broadband UVA provocation testing, and narrowband UVB testing.

Methods: Data was collected on 76 patients, of whom all had monochromator phototesting. It was examined graphically. Statistical tests used were Mann-Whitney *U* test to compare two things, Spearman tests and linear regression models. P values <0.10 were considered significant.

Results: Analysis revealed no significant relationship between objective photodiagnostic test results—including whether objective cutaneous photosensitivity or not, monochromator phototesting thresholds, UVA provocation testing responses, and narrowband UVB results—and QOL outcomes as measured by the modified DLQI, HADS, or PRIDD. Similarly, no significant connections were observed between subjective analogue scale ratings and phototesting outcomes.

Conclusion: Our findings highlight a disconnect between objective measures of photosensitivity and the self-reported impact on quality of life. This suggests that photodermatoses impose a psychosocial burden that is not adequately reflected in current phototesting results. These findings underscore the need for more holistic approaches to patient care, incorporating both clinical assessments and validated, patient-centred outcome measures.

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Feeding skin cellular regeneration: the importance of 1C metabolism and the potential of 5-MTHF to counteract UV-induced damage

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Keywords: 5-MTHF; Skin cellular regeneration; Ultraviolet radiation; Oxidative stress

One-carbon metabolism, which involves the transfer of one-carbon units via intermediates such as folate, is essential for DNA synthesis, epigenetic regulation, and the production of amino acids and lipids. The biologically active form of folate, 5-methyltetrahydrofolate (5-MTHF), plays a central role in cell growth, tissue repair, and, specifically in the skin, supports fibroblasts activity, collagen synthesis, and regeneration of damaged cells. However, solar radiation reduces skin folate levels, particularly in aging skin, impairing repair mechanisms and increasing the risk of folate deficiency [1]. While folate is well-known in nutrition, its potential in skincare—as a regenerative and anti-photoaging agent has not been widely explored. Most studies have focused on folic acid [2,3], a synthetic form of vitamin B9 that requires intracellular conversion to 5-MTHF. Due to its limited solubility, photo-instability, and need for metabolic activation, folic acid poses formulation challenges. In contrast, newly developed reduced folate salts, which are bioequivalent to active folate, show improved stability, enhanced skin penetration and antioxidant properties, making them promising candidates for cosmetic, nutraceutical and therapeutic applications [4,5].

Human dermal fibroblasts (HDF) were cultured in multiwell plates to 80-90% confluence. A time course was performed to determine the sub-lethal UVA dose for assessing cellular damage that could be counteracted by 5-MTHF (Quatrefolic®) treatment. Two experimental setups were used: pre-treatment, where cells were exposed to 5-MTHF 24 hours before irradiation, and post-treatment, where cells were irradiated first and then treated with 5-MTHF for up to 72 hours. Supplementation with 5-MTHF shows dose-dependent uptake by HDFs, remaining stable for up to 48 hours. Additionally, 5-MTHF provides significant protection against UVA-induced oxidative stress, reducing cytosolic and mitochondrial ROS production, especially at higher concentrations. This antioxidant effect improves cell viability after UVA exposure. 5-MTHF also enhances wound healing, as shown by improved closure rates in scratch assays. The photoprotective effect of 5-MTHF, both before and after irradiation, was confirmed through the comet assay, demonstrating reduced DNA damage at all tested concentrations. The impact of 5-MTHF in the remodelling of dermal structural components is also being studied.

Overall, the research points to 5-MTHF's potential as a therapeutic agent for promoting skin health, protecting against oxidative damage, and enhancing wound healing, although further investigation is required to confirm these outcomes in clinical settings.

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Thursday, August 28th

Photodynamic drug delivery: Designing liposomes for light-controlled release and enhanced chemotherapy efficacy

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Keywords: Drug delivery; Cancer; Photodynamics; Liposomes.

EXTENDED ABSTRACT

Liposomes have been the most successful type of nanomedicine for the treatment of cancer, capable of carrying chemotherapeutics with higher precision towards cancer tissues [1]. While liposomes can improve chemotherapy safety, the treatment efficacies are rarely improved due to a lack of spatioemporal control of drug release and the poor penetration of drugs into the dense cancer microenvironment. Within the field of drug delivery, photodynamics has emerged as a strikingly effective approach for spatiotemporal-controlled drug release and improved drug efficacies by harnessing photochemical redox reactions to destabilize lipid nanoformulations that contain oxidation-susceptible excipients [2].

This study outlines key design considerations for lipid nanoformulations in (1) light-controlled drug delivery, (2) overcoming lysosomal drug sequestration, and (3) facilitating microenvironmental priming to enhance tumor permeability [3]. These considerations first highlight the role of specific lipid excipients in determining photodynamic drug release efficiencies. Secondly, the selection of the photosensitizing agents is considered, which ideally absorb light >650nm and exhibit limited leaching. Thirdly, the selected photosensitizing agent and pharmaceutical cargoes may dictate which drug loading approach should be pursued, and how drug release is detected. Finally, considering the corollary effects of photodynamics on cancer cells and the cancer microenvironment, we emphasize the utility of a multi-model approach to evaluate novel photodynamic drug delivery systems.

These design considerations are intended to boost the field of photodynamic drug delivery and encourage its exploitation in translational cancer research. Moreover, these aspects are equally relevant to stimulate investigations towards oxidation-responsive drug release triggered by alternative external stimuli such as X-rays [4], thereby broadening the drug delivery arsenal against cancer.

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Porphyrin-peptide targeting systems for PDT applications

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Keywords: PDT, porphyrin, peptide, targeting

Cancer is a global severe health problem and the second leading cause of death, after heart disease[1]. In women, the overall incidence of cancer has been increasing in recent years after nearly two decades of stability[2]. Breast cancer is the second[3] most common cause of cancer death and its incidence is increasing worldwide[4].

In this context, photodynamic therapy (PDT), a therapeutic alternative that combines light and drug (photosensitizer, PS), represents a valid alternative to impact the incidence rate of cancer deaths. The latest generation of PSs are conjugated with organic and inorganic polymers, nanoparticles, liposomes, monoclonal antibodies, peptides/proteins, carbohydrates, and many others[5]. The main reason for the development of this further generation is related to the desire to direct the PSs to the target areas to maximize their selectivity and specificity and, consequently, also their intracellular accumulation[6].

In this presentation, attention will be given to some examples of PS belonging to porphyrin family bound to peptides to have a more targeted therapy. Specifically, attention will be paid to the case of Triple-Negative breast cancer (TNBC), which is defined as a subtype of breast cancer in which the expression of the proteins ER, PR and HER2 is lacking[7,8]. According to data available in official registries, breast cancer has the highest incidence in women while TNBC represents approximately 10%-15% of all breast cancers[9].

Therefore, seeking a therapy that can be targeted to this type of pathology is very important and represents a very interesting challenge[10,11].

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Cancer-Associated fibroblasts impact in Photodynamic therapy response through intercellular communication in Oral and Cutaneous Squamous Carcinoma cells

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Keywords: cancer-associated fibroblasts, photodynamic therapy, resistance, oral squamous cell carcinoma, cutaneous squamous cell carcinoma

Photodynamic therapy (PDT) offers a minimally invasive and cosmetically favorable approach for treating squamous cell carcinomas of the skin (cSCC) and oral cavity (OSCC). However, therapeutic resistance remains a major obstacle. A critical component contributing to the lack of response to the treatment is the tumor microenvironment, particularly cancer-associated fibroblasts (CAFs). Tumor cells establish signaling dialogues with fibroblasts to create an ideal environment for cancer progression and response to treatments.

This study evaluated the role of CAFs in modulating PDT efficacy in both cSCC and OSCC models. On the one hand, cSCC monolayer *in vitro* models revealed that CAF-derived TGFβ1 contributes to PDT resistance through the TGFβ1/SMAD signaling axis [1]. In OSCC, three-dimensional spheroid cultures comprising tumor cells co-cultured with patient-derived CAFs were used to mimic tumor lesions, representing in a better way real tumors characteristics. CAFs significantly increased resistance to Temoporfin-mediated PDT [2]. We also observed that conditionate medium from tumor cells reprograms fibroblast by reducing the expression of markers involved in cell death, such as FAP-1/PTPN13. This could also lead to tumor resistance, suggesting that tumor cells educate fibroblasts to create better conditions for cancer progression.

In conclusion, the dialogue between tumor cells and CAFs plays a central role in PDT in both cSCC and OSCC. Targeting the CAF–tumor cell axis emerges as a promising strategy to potentiate PDT efficacy in squamous cell carcinomas.

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Systematic Targeting of GD2-positive Neuroblastoma Tumors with Photooncolytic Phage Nanobots

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Keywords: M13; Rose Bengal; oligothiophene; scFv

EXTENDED ABSTRACT

Neuroblastoma (NB) is one of the most common pediatric neuroendocrine tumors originating from neural crest-derived sympathoadrenal precursor cells and accounts for 15% of pediatric cancer mortalities [1]. Disialoganglioside-GD2 is a key molecular target for NB immunotherapy which is based on the employment of GD2-targeting antibodies. However, about 50% of treated patients can experience tumor relapse due to limited immune-mediated cytotoxicity and poor antibody penetration into tumors [2]. To address this problem, we developed a penetrating photooncolytic phage nanovector platform that selectively targets GD2-expressing NB cells. The phage bioconjugates were functionalized with photosensitizers, and resulted in targeted oncolysis of GD2-positive NB cells upon light irradiation, without affecting GD2-negative ones. The photooncolytic phage nanobots were shown to deeply penetrate into GD2-positive tumor spheroids in vitro, and to cross biological barriers in a Zebrafish xenograft model, maintaining their ablation specificity upon irradiation. Finally, to counter resistance from GD2 loss, often linked to poor prognosis [3], we introduced a CRISPRa strategy to reactivate GD2 expression in GD2-negative cells. This approach offers a minimally invasive and highly effective strategy, addressing unmet needs in Neuroblastoma therapy.

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Sensing melanoma cells with hyaluronic acid decorated magnetoplasmonic nanoassemblies: from diagnosis to future therapeutic applications

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Keywords: Magnetoplasmonic nanoassemblies; Hyaluronic acid; Melanoma cells; Sensing; Liquid biopsia

EXTENDED ABSTRACT

Melanoma is the deadliest form of skin cancer and one of the most aggressive cancers whose incidence rates are rising in western populations [1]. The aggressive metastatic trend is associated with increased cellular dissemination. Moreover, melanoma cells secrete extracellular vesicles (EVs) thus regulating the immune cell functions and modifying the tumor microenvironment as mediators of metastasis [2]. Recently, several innovative and targeted therapies have emerged and new and reliable methods for monitoring disease progression and guiding therapeutic decisions are needed. Liquid biopsy emerges for its versatility in tailoring the response to specific biomarkers [3]. Here, a multicomponent nanoplatform for sensing melanoma cells and those associated EVs is proposed. Luminescent hyaluronic acid decorated magnetoplasmonic/amphiphilic cyclodextrin nanoassemblies were prepared and characterized using spectroscopic (FT-IR, UV/Vis, luminescence), imaging (TEM) and DLS techniques. The nanoassemblies showed no cytotoxicity up to 24 h and at 100 µg/mL on THP-1 human cell line differentiated into macrophages. The nanoassemblies were selectively uptake by A375 and HT-144 human melanoma cell lines with respect to NHDF human fibroblast cell line. After experiments on adhesion cultures, the activity was confirmed in suspension also evidencing the magnetic separation capability of the system. Finally, preliminary tests showed the interaction between the magnetoplasmonic nanoassemblies and EVs derived from a model microalgae, and from A375 and HT-144 cell lines. Results suggest that complex binding and uptake mechanisms occurred. These are probably guided by the selective recognition of the hyaluronic acid receptor CD-44 that is overexpressed in these cellular lines. Further experiments are on-going to increase the complexity of the environment for liquid biopsy applications. The theranostic potentialities are also considered.

Acknowledgement: This work is supported by the MUR-PNRR through the NextGenerationEU program with the project SAMOTHRACE "SiciliAn MicrOnanoTecH Research And innovation CEnter".

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Enhancing Tumor Uptake of Silicon Phthalocyanine and Improving PDT Efficacy in a Triple-Negative Breast Cancer Murine Model via In Situ Sonoporation

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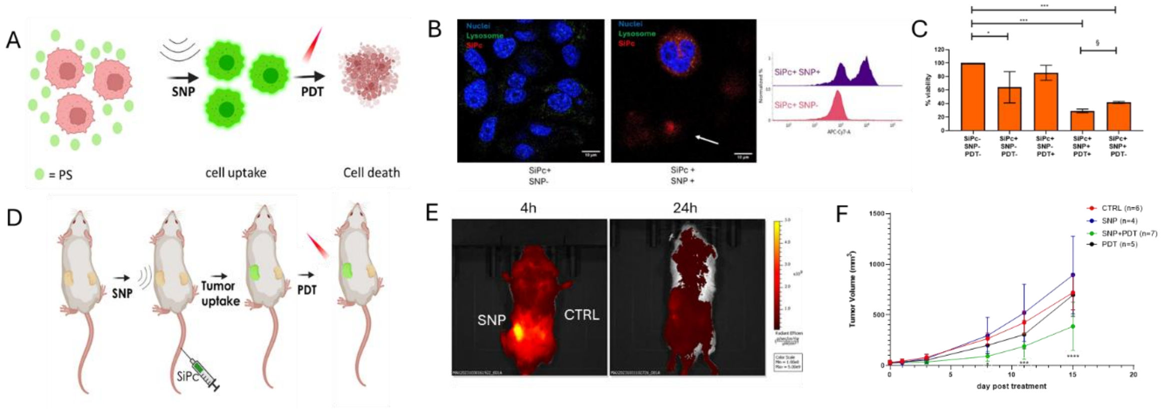
Keywords: Photodynamic therapy, breast cancer, Near infrared PDT Drug delivery, Phthalocyanine

Photodynamic therapy (PDT) effectiveness has been well demonstrated in vitro, but in vivo studies have only shown a delay in tumour growth. Tumour recurrence is often reported in clinical trials and is often associated with limited tumour uptake of the photosensitizer (PS). In this study, sonoporation (SNP) is utilized as a physical targeting tool to enhance the uptake of an untargeted PS in cells and tumour tissues. Using a chemometric approach, we identified the optimal sonoporation stimulus to maximize uptake and cell viability (frequency 1.05 MHz, tON (percentage of positive signal in a single pulse): 50%, DC: 90%, burst: 1 second, sonoporation time 1 min). We achieved a viability of 83.9% ± 10.3, and an uptake in lived cells of 53.1% ± 6.6. The combination of sonoporation and photodynamic therapy resulted in a significant reduction in cell viability. In a triple-negative breast cancer model, sonoporation combined with photodynamic therapy significantly inhibited tumour growth. For the first time, our results highlight the potential of sonoporation as a non-invasive method to increase the intratumor uptake of photosensitizers, offering a promising strategy to improve PDT efficacy.



SNP and PDT result. A) K562 cells are incubated with phthalocyanine, sonoporated, and irradiated; B) Confocal images of sonoporated and non sonoporated cells; C) MTT vitality test of K562 incubated with 1μM of SiPc D) In vivo 4T1-tumor bearing mice administered with SiPc, sonoporated and irradiated E) optical imaging 4h and 24h post sonoporation and administration F) Tumor volume reduction after combination of SNP and PDT

Fully reversible control over DNA-intercalation with visible light

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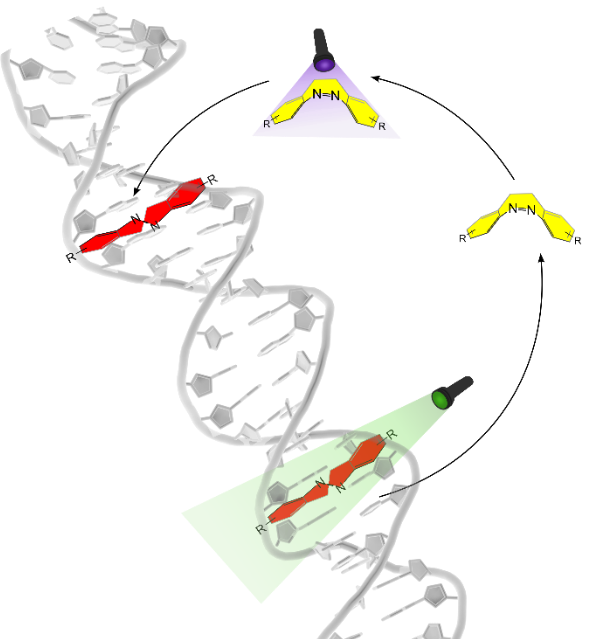
Keywords: Photopharmacology, DNA-intercalators, Photoswitches

DNA-binding agents are widely used chemotherapeutics in cancer therapy. Many established drugs, such as Cisplatin, share one intrinsic obstacle, the treatment comes with severe side-effects due to low specificity.[1] This prevalent problem in cancer therapy can be mitigated by employing the emerging concept of Photopharmacology, where the activity of a drug can be spatiotemporally controlled using non-harmful visible light.

We introduce Diazocine photoswitches (cyclic Azobenzenes) as a promising scaffold for reversible DNA-intercalation in photopharmacological cancer therapy. Diazocines are stable in *cis*-configuration and can be isomerized to *trans*-configuration by irradiation with blue light (400 nm). The metastable *trans*-Isomer can revert to the *cis*-state thermally or by irradiation with green light (535 nm). The difference in DNA binding behaviour can be explained by the change in geometry upon switching. While the flat *trans*-Isomer can intercalate between the base pairs, the sterically demanding, angled *cis*-Isomer is expelled from the DNA-Diazocine complex.



In our study, we synthesized a library of potential Diazocine based DNA-intercalators based on the results of Molecular Dynamics screening. All molecules were photochemically characterized under physiological conditions and the binding to genomic DNA was assessed with Circular-Dichroism Spectroscopy.

Our compounds show full reversibility of DNA-Binding upon irradiation and could do so without fatigue for at least 5 cycles.

The large change in affinity combined with the near-quantitative photoconversion of the Diazociness isomers lead to highly selective binding and unbinding over a wide concentration range, promising better targeting for chemotherapeutics.

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Lighting Up the Hypoxic Tumor: Folate-targeted, oxygen-generating nanoparticles for synergistic photodynamic and chemotherapeutic treatment

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Keywords: cancer; photodynamic therapy; nanotechnology; hypoxia

ABSTRACT

Hypoxia represents a major barrier to the success of many anticancer therapies, particularly photodynamic therapy (PDT) and chemotherapy. The lack of oxygen within solid tumors impairs the generation of reactive oxygen species (ROS), a critical step in PDT, and simultaneously induces metabolic adaptations that reduce tumor cell sensitivity to chemotherapeutic agents. To address these challenges, this work presents the development and evaluation of two folate-targeted, oxygen-generating nanoparticle systems designed to restore oxygen availability and enable a synergistic therapeutic response. Both nanosystems are based on human serum albumin (HSA) nanoparticles co-loaded with a chemotherapeutic prodrug, paclitaxel (PTX_2-SXS), and the photosensitizer chlorin e6 (Ce6). Surface functionalization with folic acid (via DSPE-PEG-FA) enables selective targeting of tumor cells overexpressing folate receptor alpha (FRα). The two formulations differ in their strategy to overcome hypoxia: catalase (Cat) for the conversion of endogenous hydrogen peroxide found in the tumor microenvironment into molecular oxygen or hemoglobin (Hb) as an oxygen carrier and manganese oxide-functionalized albumin (mHSA), which mimics catalase activity and contributes to sustained oxygen generation. These nanoparticles were thoroughly characterized for their biological properties: folate-driven cellular uptake, subcellular localization, and ROS production following red light activation (600–780 nm). Their cytotoxic potential was evaluated in two cancer cell lines (OVCAR-3 and SK-OV-3), differing in FRα expression levels. Key outcomes included analysis of dark and photo-induced cytotoxicity, intracellular oxygen release under hypoxic conditions, and the resulting cell death mechanisms. The results demonstrate that these multifunctional, actively targeted nanocarriers effectively reoxygenate hypoxic tumor environments and enhance the cytotoxic synergy between PDT and chemotherapy. This dual-action nanoplatform offers a promising strategy to "light up" hypoxic tumors and overcome their resistance to conventional treatments.

Multiscale time resolved spectroscopy to decipher the photo-activation mechanism of a switchable near-infrared fluorescent bacteriophytochromes.

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Keywords: ultrafast; spectroscopy; protein fluorescent; photochromism

The development of novel reversible switchable fluorescent proteins (RSFPs) that work in the red/NIR domain is one of recent challenges in nanoscopy as this domain is highly advantageous for live-cell imaging due to its reduced phototoxicity, lower autofluorescence, greater penetration depth in vivo, and expanded spectral multiplexing capabilities. Another key challenge is also the need for fast RSFPs with sub-second thermal back recovery of the Off state as it enables bio-applications with only a single wavelength [1].

Here, we present the development of fast NIR RSFPs based on a derivative of the wild-type bacteriophytochrome from Deinococcus radiodurans (Dr-PSM) [2]. The stable form of Dr-PSM absorbs in the red (P_r, ON state) and, upon irradiation, converts into a form that absorbs in the near-infrared (P_{fr}, OFF state). While Dr-PSM OFF state thermal recovery takes several days, Stefan Jakobs' group has developed a derivative, PENELOPE, the first RSFP to function in Near Infrared nanoscopy with an in vitro millisecond thermal recovery.

Developing new mutants based on PENELOPE requires a deep understanding of its photoswitching mechanism, particularly to identify the species that control its fluorescence and switching quantum yields, as well as its rapid thermal recovery. The photoswitching from P_r to P_{fr} in wild-type bacteriophytochromes involves multiple processes, including a picosecond cis-trans isomerization of the biliverdin chromophore, millisecond deprotonation and reprotonation steps, and structural changes in the protein, leading to the formation of the final P_{fr} state within hundreds of milliseconds. The photodynamics thus involves several excited and intermediate states, with lifetimes spanning fifteen orders of magnitude, from femtoseconds to seconds.

The photodynamics of PENELOPE was studied using multiscale time-resolved optical spectroscopy, including fluorescence and transient absorption UV-Vis-NIR, covering a time range from femtoseconds to seconds. A comparison was made with variants of the wild-type protein exhibiting accelerated thermal recovery or fluorescence without photoswitching capabilities. This approach allowed us to reveal key intermediates that govern switching quantum yield and thermal back recovery. We will discuss here our findings and their implications for the design of novel NIR RSFPs optimized for nanoscopy applications.

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How hydroxylated carotenoids enable ultrafast energy transfer in the Kin4B8 rhodopsin: A multiscale computational study

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Keywords: Rhodopsin–carotenoid complexes; Light-harvesting; Spectroscopic simulations; Excitation energy transfer (EET)

Rhodopsin–carotenoid complexes represent an emerging class of microbial light-harvesting systems that broaden the spectral range of phototrophy beyond conventional retinal-based absorption.[1] While 4-ketocarotenoids were traditionally believed to be essential for excitation energy transfer (EET), recent experimental studies have challenged this view, demonstrating that hydroxylated carotenoids such as zeaxanthin and lutein can also support efficient EET to retinal chromophores.[2]

In this talk, I will present a multiscale computational study that reproduces these experimental observations and provides a mechanistic, atomistic-level understanding of EET in the Kin4B8[2] rhodopsin. Our approach integrates molecular dynamics, hybrid quantum mechanics/molecular mechanics (QM/MM) simulations, and excitonic modeling[3] to investigate the structural and electronic factors underlying excitation energy transfer.

We find that hydroxylated carotenoids preferentially bind via their β-ring within a conserved protein fenestration, stabilized by a specific hydrogen-bonding network. This binding geometry enables strong excitonic coupling between the carotenoid S_2 and retinal S_1 states, facilitating ultrafast EET with ~70% efficiency on sub-100 fs timescales, consistent with ultrafast spectroscopic data.[2] Additionally, our simulations show that the characteristic biphasic circular dichroism signal arises from a cooperative mechanism involving both excitonic interactions and induced chirality of the retinal and carotenoid chromophores. Enhanced sampling molecular dynamics further highlights the functional role of a glycine residue located near the β-ionone ring of the retinal; its mutation to phenylalanine disrupts carotenoid binding and abolishes energy transfer.

These findings reveal that efficient light harvesting in rhodopsin–carotenoid systems is driven not by specific carotenoid functional groups, but by precise protein–pigment structural complementarity. This work provides a theoretical framework for interpreting new experimental insights and offers principles for the rational design of biomimetic light-harvesting architectures.

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Integrated structural dynamics of a vitamin B12-dependent photoreceptor

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Keywords: time-resolved structural biology, photoreceptor

Photoreceptor proteins use light absorption to elicit a biological response in various processes such as vision, circadian rhythms and plant development. How the initial photochemical events at the receptor's chromophore funnel through space and time into the desired photobiological event is a focus of intense current research.

We focus on the CarH photoreceptor, an archetype of the newly discovered superfamily of B_{12} dependent photoreceptors that surprisingly repurpose and finely tune the ubiquitous vitamin B_{12} cofactor to sense light. CarH is a tetrameric light-responding repressor of carotenoid gene expression. Upon light-activation, structural changes lead to disassembly of the tetramer and its release from DNA to allow gene transcription. We combined time-resolved serial femtosecond crystallography at XFELs and time-resolved X-ray solution scattering at a synchrotron to study structural changes on the ns to s time-scale that lead from cleavage of the photolabile chromophore bond to tetramer dissociation. Complementing the time-resolved structural studies with results from cryo temperature-controlled X-ray crystallography and *in crystallo* absorption spectroscopy, from various time-resolved and temperature-controlled spectroscopies and from QM/MM calculations allowed providing and connecting essential pieces in the puzzle of B_{12} dependent photoreception.

The relevance of amino acid-chromophore interaction in protein conformational changes and their impact on chromophore relaxation dynamics

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Keywords: BioHybrids, chromophores, protein engineering, photoactivity

In natural photosynthesis, light energy is transferred through a dynamic protein-pigment network with remarkably high quantum efficiency. Local fluctuations in protein structure and conformational changes play a crucial role in shaping the chromophore's energy landscape for efficient light harvesting. However, the precise mechanistic role of these conformational changes remains unclear due to the complexity of natural photosystems, making them difficult to control. Photoactive biohybrids (Fig.1)—synthetic systems that incorporate the key components of natural photosystems, such as proteins and chromophores—offer a promising approach for applying these natural principles in man-made technologies[1]. However, designing these biohybrids presents significant challenges, particularly in controlling the protein-chromophore interaction network, which unpredictably affects the electronic and conformational states of the chromophores[2]. In our lab, we have developed biohybrids based on optimized de novo proteins with well-defined cavities and flexible conformational states[3]. This design enables precise tuning of the biohybrid's response through single-point mutations. Our biohybrids provide a powerful platform for studying the fundamental role of protein amino acids in chromophore relaxation, offering new insights for bio-inspired nanotechnology and photonics.

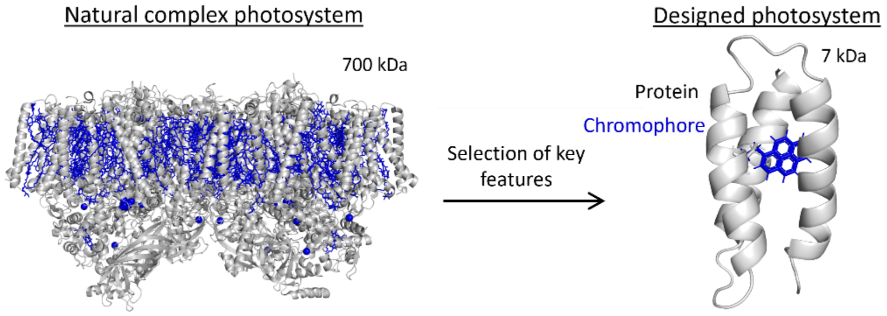

Figure 1. Simplified photosystems to study the mechanistic role of protein amino acids in chromophore relaxation dynamics.

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Electron Return Kinetics in Successive Two-Photon DNA Repair by (6-4) Photolyase

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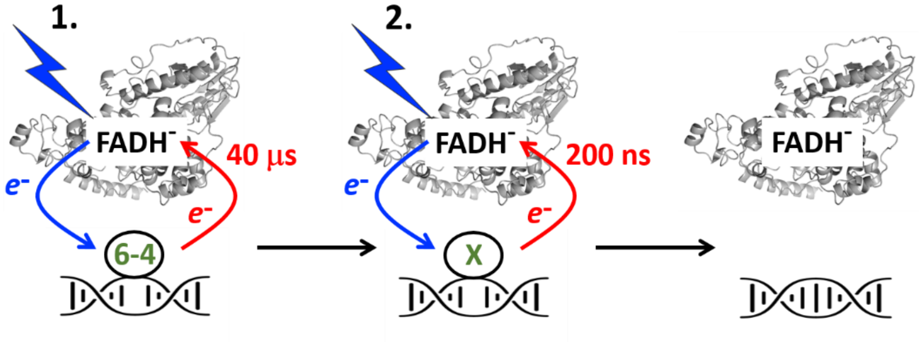
Keywords: DNA repair, (6-4) photolyase, electron transfer, transient absorption spectroscopy

(6-4) photolyase is a flavoenzyme that catalyzes the chemically challenging repair of UV-induced carcinogenic (6-4) photoproduct lesions in DNA using sunlight. Nature appears to have overcome the challenge through two distinct successive photoreactions, each initiated by electron transfer from the excited, fully reduced flavin cofactor ($FADH^-$) to the DNA lesion.

Here we present a transient absorption study[1] of electron returns to the flavin following the chemical transformations of the lesion. We successfully resolved the kinetics of electron returns associated with the completion of the first and second photoreactions (~40 µs and ~200 ns, respectively) in the (6-4) photolyase from *Xenopus laevis*, corroborating and detailing the two-photon reaction model[2].



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Time-resolved structural dynamics of natural and engineered photoreceptors containing non-canonical amino acids

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Keywords: Genetic code expansion; Ultrafast spectroscopy; Vibrational probes; Protein photocontrol

Time-resolved Raman [1] and infrared [2] spectroscopies can provide kinetic and structural information down to the femtoseconds time scale. However, two features hinder a broader application of transient vibrational spectroscopy in protein photobiology. First, overlapping bands typically prevent selective investigation of native protein bonds. Second, the use of UV/Visible laser pulses to excite a chromophore and start a reaction severely limits the number of target systems. Here, we employ an approach to monitor and initiate photoinduced protein structural dynamics based on genetically encoded non-canonical amino acids (ncAA). On the one hand, we have incorporated well-known [3, 4] and novel ncAA carrying “transparent window” tags to monitor the complete photocycle of the light-oxygen-voltage (LOV) transcription factor EL222 residue-by-residue. On the other hand, genetic encoding of photocaged [5] and photoswitchable ncAA is allowing us to trigger changes in protein folding and interactions by light. Our results underscore the power of light-responsive ncAA as (i) spectroscopic reporters of protein microenvironments, and (ii) phototriggers to set proteins in motion for pump-probe spectroscopy techniques and potentially also time-resolved structural biology methods.

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Dynamics and Fate of the CO_2 Photoproduct in Fatty Acid Photodecarboxylase

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Keywords: Fatty Acid Photodecarboxylase; Photobiocatalysis; Time-Resolved spectroscopy

Fatty acid photodecarboxylase (FAP) is an algal photoenzyme that converts fatty acids into hydrocarbons via a light-driven radical mechanism[1]. Previous cryogenic FTIR and crystallographic studies suggested that a significant fraction of the CO_2 byproduct is enzymatically transformed into bicarbonate through interaction with water within the active site[2]. We aimed to clarify whether this bicarbonate intermediate persists at physiological temperature and to determine the fate of CO_2 in FAP's photocatalytic cycle.

We combined steady-state and time-resolved infrared spectroscopy (FTIR at 100–200 K; TRIR at 298 K), membrane-inlet mass spectrometry (MIMS) with $H_2{}^{18}O$ and ^{13}C-labeled substrates, site-directed mutagenesis (R451K, C432S), isotope-exchange experiments, and quantum-chemical (QM/MM) simulations to track CO_2 formation, conversion, and release.

Cryogenic FTIR confirmed bicarbonate formation at 150–200 K in wild-type FAP, which is absent in R451K and C432S mutants and confirming the roles of residues R451 and C432 in low-temperature CO_2 reactivity. However, TRIR and MIMS at room temperature showed neither a concurrent release of $^{13}C^{16}O^{18}O$ with $^{13}C^{16}O_2$ nor any transient HCO_3^- bands; MIMS data instead indicated that CO_2 reacts with solvent post-release under these conditions. QM/MM calculations revealed that the bicarbonate formation barriers increase dramatically at 298 K and that C432 can rapidly reprotonate R451, preventing stable bicarbonate trapping.

Although bicarbonate is stabilized in FAP under cryogenic conditions, it does not appear as a catalytic intermediate at physiological temperature. Instead, CO_2 exits the active site and reacts with water only in solution. These findings revise the mechanistic model of FAP, highlighting an as-yet unidentified in-protein CO_2 transformation pathway.

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Multi-step 11-cis to all-trans retinal photoisomerization in bestrhodopsin, an unusual microbial rhodopsin

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Keywords: rhodopsin; femtosecond stimulated Raman spectroscopy; isomerization; multi-timescale

Rhodopsins constitute a broad class of retinal-binding photoreceptors. Microbial rhodopsins are canonically activated through an all-trans to 13-cis photoisomerization, whereas animal rhodopsins are mostly activated through an 11-cis to all-trans isomerization. Bestrhodopsins constitute a special microbial rhodopsin subfamily, with bistable rhodopsin domains that can be photoswitched between a far red-absorbing state D661 and a green-absorbing state P540. Its photochemistry involves a peculiar all-trans to 11-cis isomerization for the D661 to P540 photoreaction and vice versa. Here, we present the P. antarctica bestrhodopsin 11-cis to all-trans photoreaction as determined by femtosecond-to-sub-millisecond transient absorption, femtosecond stimulated Raman and flash-photolysis spectroscopy. The primary photoreaction involves ultrafast isomerizations in 240 fs from the 11-cis reactant to a mixture of highly distorted all-trans and 13-cis photoproducts. The 13-cis fraction then thermally isomerizes to a distorted all-trans RSB in 120 ps. We propose bicycle pedal models for the branched photoisomerizations with co-rotation of the C11=C12 and C13=C14 double bonds. One reactant fraction undergoes bicycle pedal motion aborted at the C13=C14 double bond, resulting in all-trans retinal. The other fraction undergoes a full bicycle pedal motion of both C11=C12 and C13=C14, resulting in 13-cis retinal. The primary products are trapped high up the ground state potential energy surface with a low energetic barrier that facilitates thermal isomerization from 13-cis to all-trans retinal in 120 ps. All-trans retinal then structurally and energetically relaxes with subsequent time constants of 0.7 and 62 μs and 4.4 ms along with counterion protonation, completing the P540 to D661 photoreaction.

Conformational heterogeneity and protonation equilibria shape the photocycle branching in channelrhodopsin-2

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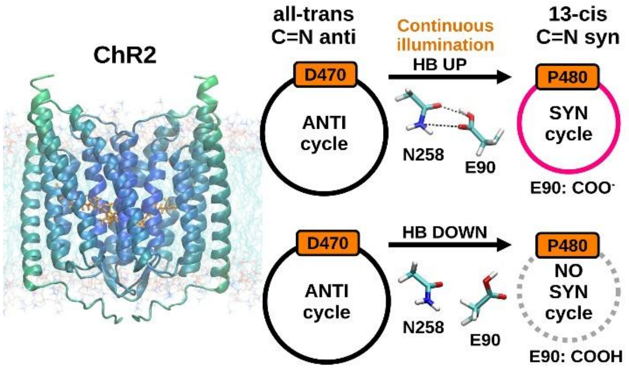
Keywords: Optogenetics, ChannelRhodopsin2, Molecular Dynamics, Quantum Mechanics



The emerging field of optogenetics exploits the genetic encoding of photoactive proteins to control physiological processes with high spatiotemporal resolution and in a wavelength-dependent manner. Microbial rhodopsins serve as primary optogenetic tools due to their ability to regulate neuronal activity via light-gated ion channels or light-driven pumps. Channelrhodopsin2 (ChR2) is the most used optogenetic tool and a widely investigated rhodopsin. Upon light absorption, the retinal cofactor undergoes photoisomerization from the all-trans (D470 state) to the 13-cis configuration (P500 state). This conformational change induces the flow of water and ions through the channel on the microsecond timescale, followed by the recovery of the closed resting state within milliseconds. However, despite extensive experimental studies, many structural modifications associated with photocycle intermediates remain debated [1]. Of particular interest is the localization of the P480 intermediate within the photocycle, which may involve a C=N anti/syn isomerization, as well as the timing of the deprotonation of glutamic acid E90, a critical residue for ChR2 function. In this study, we investigate the possibility of an early-P480 state, formed directly upon photoillumination of the dark-adapted state, in which E90 is deprotonated, as hypothesized in a previous work [2]. Employing extended molecular dynamics simulations and QM/MM multiscale calculation based on the Perturbed Matrix Method [3], we computed the deprotonation free energy and the infrared band associated with E90, supporting the existence of the early P480 state [4]. Our findings also show that both protonated and deprotonated E90 states are possible in P480, depending on E90's hydrogen bonding pattern, explaining the molecular mechanism underlying P480 accumulation under continuous illumination. By establishing robust correlations between structural changes and infrared spectral features in ChR2, our findings refine the interpretation of experimental data. This work is expected to enhance our understanding of the molecular mechanisms underlying optogenetic control, ultimately improving the precision and effectiveness of such tools in neuroscience and beyond.

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Mechanism and Dynamics of Photoswitchable Flavoprotein Charge-Transfer Complexes

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Keywords: Photoswitches; Flavoproteins; Time-resolved spectroscopy; Charge-transfer complex

Because of their diverse applications in biological science and engineering, continued efforts have been made to expand the pool of photoswitchable protein systems. Using ultrafast spectroscopy, we demonstrated that in two nonphotocatalytic flavoenzymes, monomeric sarcosine oxidase (MSOX) and *N*-methyltryptophan oxidase (MTOX), photoexcitation of a charge-transfer (CT) complex formed by a flavin adenine dinucleotide (FAD) cofactor and a nonreactive ligand, e.g., methylthioacetate (MSA) or methylselenoacetate (MSeA), induced an unprecedented photochromic reaction. This reaction involved the disappearance of the CT interaction and formation of the uncomplexed FAD, on sub-picosecond timescales for the forward photoswitching, and picosecond-to-nanosecond timescales for the backward recovery, which we assigned to the photoinduced, reversible conformational changes of the ligand in the protein active site.[1,2] With further protein mutagenesis, and classical and quantum chemical calculations, we demonstrated that (1) the kinetic behaviors of the photoswitching cycle are protein- and ligand-dependent, (2) the photoswitching and backward thermal recovery rates can be tuned by mutation of a specific active-site residue (Met245 and Thr239 in MSOX and MTOX, respectively), with recovery rates spanning over an order of magnitude, and (3) modifications of the protein environment alter the conformational energy landscape of the ligand–flavin complex, consequently regulating the photocycle.[3] Taken together, these findings highlight the potential of flavoprotein CT complexes in the development of fast photochromic protein systems, and provide a molecular basis for fine-tuning their photophysical properties.

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UVR8 photoreceptor action in plant responses to UV-B radiation

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Exposure of plants to UV-B wavelengths initiates a range of responses that modify metabolism, development and physiology to enable acclimation to the radiation environment. Many of these responses are mediated by the UV RESISTANCE LOCUS8 (UVR8) photoreceptor through extensive differential regulation of gene expression.

Exposure of UVR8 to UV-B and short wavelength UV-A causes dissociation of the homodimer into monomers. Monomeric UVR8 accumulates in the nucleus where it initiates signaling. UVR8 functions through interaction with other proteins. Interaction with REPRESSOR OF UV-B PHOTOMORPHOGENESIS (RUP) proteins promotes dimer re-association and represses UVR8 activity. Interaction with CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), associated with a SUPPRESSOR OF PHYA-105 (SPA) protein, prevents COP1 from targeting the ELONGATED HYPOCOTYL 5 (HY5) transcription factor for degradation. HY5 is a key effector of transcription of many genes regulated by UVR8 signaling. In addition, UVR8 interacts directly with various transcription factors, modifying their ability to regulate sets of genes involved in specific UV-B responses.

Although these interactions are crucial in enabling UVR8 to initiate responses, the mechanisms responsible for differential protein interaction are not fully understood. Potential mechanisms involved in the regulation of UVR8 action will be discussed. In particular, our recent research has identified phosphorylation of UVR8 as an important regulatory mechanism.

UV-B perception and signaling in plants

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Keywords: Photomorphogenesis; Photoreceptor; Signal Transduction; UV-B

Plants perceive UV-B radiation through the evolutionarily conserved photoreceptor UV RESISTANCE LOCUS 8 (UVR8) [1]. In its inactive state, UVR8 forms a homodimer, which monomerizes upon UV-B absorption via specific intrinsic tryptophan residues. The resulting active UVR8 monomers interact with the E3 ubiquitin ligase CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), initiating a signaling process that alters gene expression. This leads to a broad range of UVR8-dependent physiological responses, including those that support UV-B acclimation and stress tolerance. The pathway is attenuated by the WD40-repeat proteins REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1) and RUP2, which promote UVR8 redimerization and dissociation from COP1. I will present recent insights into the evolutionary trajectory of this UV-B signaling network in plants, from initial photoreception to downstream responses that enhance survival under sunlight.

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Chloroplast movements in alpine plant species along an elevational gradient in UV radiation in the Tatra Mountains

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Abstract:

Chloroplast movements serve a photoprotective function in plants. They are controlled by UV/blue light photoreceptors called phototropins. The quality and quantity of light, as well as temperature, modulate the magnitude of chloroplast responses. We used a natural elevational gradient in UV radiation in the Tatra Mountains to investigate how chloroplast movements contribute to photoprotection in four habitats. Sites differing in habitat and solar irradiance with elevation (1080-1975 m asl) were chosen to examine leaf physiological traits. Among three alpine species, leaves of *Vaccinium myrtillus* showed robust chloroplast movements, with a high amplitude of chloroplast avoidance found at the highest-elevation alpine site. The maximum photosynthetic yield of photosystem II did not change with elevation, implying that *V. myrtillus* is adapted to a wide ecological niche. Despite having thick leaves, *Homogyne alpina* displayed effective chloroplast movements. In *Soldanella carpatica* leaves, only small changes occurred in transmittance resulting from chloroplast avoidance. This suggests that other mechanisms, e.g., flavonoid accumulation, were likely important for acclimation to high irradiances at high elevations. For all species, the magnitude of transmittance changes due to chloroplast avoidance was inversely proportional to leaf thickness. The magnitude of chloroplast responses relied mainly on the local light conditions, differing with habitat, rather than elevation.

UV-B-mediated acclimation of Norway spruce and Scots pine to high light stress depends on photoperiod

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Keywords: conifer photobiology, UV-B radiation and high-light, photoperiod, acclimation and signaling

Light is a critical abiotic factor for plant growth and development, since it drives photosynthesis and regulates multiple physiological and biochemical processes that allow plant acclimation to the changing environment. Ultraviolet B (UV-B, 280 – 315 nm) radiation is a small but biologically significant component of the solar spectrum that at low levels can help plants mitigate UV-induced damage and acclimation to other stress factors [1]. These UV-B induced acclimatory responses are largely mediated by the UVR8 pathway [2] and are well characterized in model plant species such as *Arabidopsis thaliana* and crops [1, 3], but not in conifers. In this study, we assessed the effects of UV-B radiation on physiological and molecular responses of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* (L.) to high-light (HL) stress. A multifactorial experiment was designed where both species were grown for two months under different photoperiods (8h or 12h of light) using visible light (400 – 700 nm, 150-170 µmol/m^{2}s). Afterwards, plants were pre-treated with two different fluence rates of UV-B provided by narrowband UV-B fluorescent tubes either low UV-B (2.9 µmol/m^{2}s) or high UV-B (4.9 µmol/m^{2}s) for 3h per day over a period of 1 or 2 days, and subsequently exposed to 1h of HL stress (1000 µmol/m^{2}s of visible light). Arabidopsis wild type Col-0 and the null UVR8 mutant, *uvr8-6*, were included as internal controls in the experiment. OJIP curves, photosynthetic efficiency (Fv/Fm), and antioxidant capacity including catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), and peroxidase (POD) activities were measured simultaneously with the accumulation of transcripts of genes involved in UV-B acclimation. Photosynthetic performance was significantly influenced by the UV-B pre-treatment, the photoperiod, and the HL stress. Furthermore, the Fv/Fm response to the UV-B pre-treatment differed in Norway spruce compared to Scots pine independently of the photoperiod. A two-day exposure to high UV-B pre-treatment enhanced acclimation to subsequent HL stress in both species, preserving photosynthetic capacity after HL stress compared to plants exposed to low UV-B and control conditions. This HL acclimation response involved the regulation of CAT and APX activities, as well as differential transcript accumulation of *ELONGATED HYPOCOTYL 5 (HY5), CHALCONE SYNTHASE (CHS),* and *REPRESSORs OF UV-B PHOTOMORPHOGENESIS* (*RUPs)*, which also exhibited significant species- and photoperiod-dependent trends. Data from Arabidopsis confirmed that the molecular and physiological responses to the UV-B treatments used in the experiment were mediated via the UVR8 signaling pathway. Together, this study advances our understanding of UV-B-induced signaling in conifers and opens the door for future use of UV-B radiation in plant production practices to enhance seedling resilience to HL stress.

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Transcriptional responses to UV-B radiation in Arabidopsis are shaped by PAR intensity and genetic variation

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Keywords: Acclimation; Arabidopsis; Natural variation; UV-B radiation

Ultraviolet radiation (UV, 280–400 nm) is a key environmental cue that influences plant growth, development and metabolism. Plants detect ambient levels of UV-B radiation (280–315 nm) through the UVR8 photoreceptor which orchestrates changes in gene expression leading to UV-B acclimation. However, plant responses to UV-B are strongly influenced by photosynthetically active radiation (PAR, 400–700 nm), and the mechanisms through which PAR modulates UV-B responses remain poorly understood. This study used natural variation in *Arabidopsis thaliana* to explore the molecular and physiological basis of UV-B responses. We aimed to identify transcriptional patterns and genetic regulators involved in light acclimation by analyzing three Arabidopsis accessions with varying UV-B sensitivities: C24 (sensitive), Col-0 (intermediate) and Ga2 (tolerant).

Plants were grown under two PAR levels (150 or 300 μmol m^{-2} s^{-1}) for two weeks, then exposed to narrowband UV-B radiation (4.6 μmol m^{-2} s^{-1}) for four hours. This created two distinct UV-B:PAR ratio conditions: high (4.6:150) and low (4.6:300). We also assessed the effects of PAR intensity alone by comparing plant responses across the two PAR levels. RNA-seq analysis revealed accession-specific transcriptional responses, with principal component analysis separating accessions more clearly than treatment groups. Gene Ontology enrichment highlighted strong condition-specific responses, especially in Col-0 under high UV-B:PAR, where genes linked to transcription, RNA biosynthesis, and stress response were enriched. In contrast, high PAR alone elicited modest activation of ribosome biogenesis and RNA processing pathways. Flavonoid accumulation in the adaxial epidermis also varied by accession. C24 increased flavonoids only under low UV-B:PAR, whereas Col-0 and Ga2 showed UV-B induced flavonoid accumulation under both PAR conditions. Our findings demonstrate that transcriptional and metabolic responses to UV-B in Arabidopsis are shaped by both genetic background and visible light environment.

Screening overrules damage repair: layers of protection for enhanced UV tolerance in *Botrytis cinerea*

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Melanin; photolyase; gray mold; postharvest

Gray mold caused by cryptic species of *Botrytis* can lead to devastating pre and postharvest losses if not properly managed. Use of short wavelength ultraviolet (UV) radiation can be one among the non-chemical alternatives in control of gray mold. Previous studies showed less efficiency of UV against developmental stages of *Botrytis cinerea*, pigmented fungi causing gray mold compared with *Oidium neolycopersici*, non-pigmented fungi causing powdery mildew in tomato. Experiments were conducted at Petri dish level to examine the contribution of melanin (and their intermediates) mediated UV screening, and photolyase-UV endonuclease mediated damage repair in relation to enhanced tolerance of *B. cinerea* to UV. Reference *B. cinerea* isolate B05.10 was cultured in solid complete nutrient medium (CM) for 14 days with continuous lighting of 60±10 µmol $m^{-2}s^{-1}$ provided with cool white, fluorescent lamps. Colonies with proficient sporulation were selected and washed by adding sterilized distilled water followed by gentle pipetting. The resulting suspension was filtered and used as conidial suspension for experiments. Deletion mutant genotypes were developed using protoplast transformation mediated homologous recombination strategy (Chung and Lee, 2015). Knock out mutants of target genes of white collar (wc1, wc2), photolyase (phr1-1), UV endonuclease (uve28), melanin mutants of polyketide synthase 13 (pks13), scytalone dehydratase 1 (scd1-3) and double mutants of wc1-pks13, uve28-phr1-1 were generated as described previously (Zhu, P et al 2018). Sterilized pieces of cellophane overlay were placed in Petri dishes having solid complete nutrient medium. The concentration of the conidial suspension prepared, as described above, from all genotypes was 10^5 conidia mL^{-1}. A drop of conidial suspension (20 µL) was added on cellophane overlay and allowed for air dry at room temperature under laminar air flow for 10 min. Samples were then exposed to germicidal UV radiation (peak at 254 nm) using UV fluorescent lamps (HL-2000 crosslinker lamps, UVP, USA) with an irradiance of 13 Wm^{-2} for 0, 15, 30 or 60 s. Immediately after UV treatment, Petri dishes were sealed and incubated in dark at 20±1 ˚C. Samples were examined with inverted light microscope 5 h after UV treatment. Fifty conidia were observed for visible germ-tube, and germination percentage was calculated. Images were taken using a digital camera. Among the tested genotypes, melanin mutant of *pks13* showed least tolerance to UV even at the lowest exposure of 15 s. However, the photolyase mutant showed tolerance equal to wild. With an increased duration of exposure to 30 s, *pks13*, *uve28*, and the double mutant of *uve28-phr1-1* were equally vulnerable to UV (lowest tolerance). At the UV exposure level of 60 s, all the genotypes were equally vulnerable compared with the wild type, except *scd1-3* mutant. Melanin mediated UV screening play a dominant role in *B. cinerea* tolerance to UV than photolyase mediated damage repair. Among the damage repair mechanisms, UV endonuclease play dominant role than photolyase. Further research is necessary to examine the potential risk of UV induced development of scytalone dehydratase mutant which had greater tolerance to UV than wild.

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Physiological response of *Porphyra linearis* (Rhodophyta) to different UV radiation doses

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Keywords: Bioactive compounds; Mycosporine-like aminoacids (MAAs); Seaweeds; UV radiation

Porphyra sensu lato includes red algal strains with economic importance to the food industry, as they are a rich source of proteins, amino acids, iron, and vitamins [1]. This alga is also valuable to the pharmaceutical industry due to its bioactive compounds (BACs), such as phycobiliproteins, mycosporine-like amino acids (MAAs), polysaccharides, and polyphenols, which have beneficial effects on human health, including antiviral, anticancer, antioxidant, and photoprotective activities [2–5]. Understanding the tolerance and physiological response of *P. linearis* to ultraviolet radiation (UVR) is critical in the context of increasing UV exposure due to ozone layer depletion, and also to clarify the role of UVR in the production of economically important BACs. This study evaluated the effects of different UVR doses on the physiology of *P. linearis* under controlled laboratory conditions. Prior to the experiment, samples underwent a 5-day acclimation under 120 μmol photons$\cdot$m$^{-2}\cdot$s^{-1} of photosynthetic active radiation (PAR), a 12:12 light-dark photoperiod, 15 °C, and a simulated tidal regime of 12:12 h, with weekly supplementation of 1.5 mM KNO_3 and 0.09 mM KH_2PO_4. The experimental phase lasted 8 days under the same physical conditions, but with increased nutrient levels (5 mM KNO_3 and 0.3 mM KH_2PO_4). Algal density was maintained at 4 g$\cdot$L^{-1}. Treatments included: (i) control with no UVR; (ii) 6 h of UVR exposure at 8 W$\cdot$m^{-2} (daily dose: 172.8 kJ$\cdot$m^{-2}); (iii) 9 h UVR (259.2 kJ$\cdot$m^{-2}); and (iv) 12 h UVR (345.6 kJ$\cdot$m^{-2}). Photobiological responses and bioactive compounds (phycobiliproteins, polyphenols, MAAs, and antioxidant activity) with protective effects against harmful wavelengths, particularly UVR and blue light, were quantified on days 4 and 8. In terms of photosynthetic performance, maximal quantum yield (Fv/Fm) showed little variation between treatments; however, significant declines were observed after 8 days of exposure to 6 and 12 hours of UVR exposure. Conversely, maximal electron transport rate (ETRmax) as estimator of photosynthetic capacity increased in all 8-day treatments and in the 4-day control group without UVR. Notably, all UVR-exposed groups after 4 days presented the lowest ETRmax values. Regarding bioactive compounds, phycoerythrin showed the highest concentrations in the 8-day treatments, particularly in those without UVR or with 6 h per day of exposure. Phycocyanin concentrations were enhanced in all 8-day treatments. Total polyphenols peaked in all treatments on day 4 and remained elevated in samples exposed to 9 h of UVR at day 8. MAAs were most abundant following 6 h of UVR for 4 days, while the greatest antioxidant activity was detected in the 4-day control group without UVR exposure. These results suggest that moderate UVR exposure can stimulate photoprotective mechanisms and enhance the accumulation of valuable bioactive compounds in *P. linearis*. However, prolonged or high-dose exposure compromises photosynthetic efficiency and antioxidant capacity.

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Acknowledgment: “Funded by the European Union under the Marie Sklodowska-Curie grant agreement No. 101106349. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Commission (granting authority). Neither the European Union nor the granting authority can be held responsible for them”.

Detection of chloroplast movements through changes in leaf's ability to depolarize light: application of the Umov effect

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Keywords: chloroplast movements; reflectance; depolarization; Umov's effect

We present a sensitive method for non-contact detection of chloroplast movements in leaves and other photosynthetic tissues, based on changes in the interaction between the leaf and polarized light [1]. We examined red light reflectance of leaves during irradiation with blue light, known to trigger chloroplast relocations. Experiments on the model plant Arabidopsis thaliana, wild-type and several mutants with disrupted chloroplast movements, showed that the chloroplast avoidance response, induced by high blue light, led to a substantial increase in diffuse reflectance of unpolarized red light. The effects of the accumulation response in low blue light were the opposite. The specular reflectance of the leaf was unaffected by the chloroplast positioning. To further improve the specificity of the detection, we examined the effects of chloroplast relocations on the leaf reflectance of a linearly polarized incident beam. The greatest relative change associated with chloroplast movements was observed when the planes of polarization of the incident and detected beams were perpendicular. Further experiments revealed that the chloroplast positioning affected the magnitude of depolarization of light by the leaf. The positive correlation between leaf reflectance and its ability to depolarize light is known as the Umov effect. This phenomenon has been observed for scattering materials observed at high angles and used in astronomical studies of surface properties of extraterrestrial bodies. We applied the developed approach to examine chloroplast relocations in four angiosperm species collected in the field. The method allowed us to detect the chloroplast avoidance response in the green stems of bilberry, a sample not amenable to transmittance-based detection. Despite the importance of chloroplast movements for the optimization of photosynthetic efficiency and biomass production, high throughput reflectance-based methods are not routinely used for their detection. Characterization of polarized reflectance opens the possibility of non-invasive, non-contact detection of chloroplast relocations in a manner insensitive to the orientation of the leaf.

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Clothing in Dependence of Temperature, Gender and Age as influencing factor for Everyday Life UVR Exposure

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Keywords: UV radiation exposure; Clothing; Temperature; Gender

EXTENDED ABSTRACT

Clothing is one of the most important factors for solar ultraviolet (UV) exposure of people. However, there is little information on clothing habits available. Therefore, we investigated peoples' clothing habits in dependence of meteorological parameters during everyday life.

To that end, we developed a body chart, which divides the body into six sections, together with a coding scheme that describes the body coverage by worn garments for each section. This allows to distinguish hundreds of garment combinations. Clothing habits of around 10.000 adults were observed in the urban region of Vienna and meteorological conditions were recorded. People were divided by gender and grouped into young adults, mature adults and elderly adults.

Our study shows that air temperature (in the range of 7°C to 37°C) is the most important factor, while wind speed and humidity did not show any significant influence. With respect to clothing, significant differences in body coverage between females and males were found in all age groups: females have lower body coverage at temperatures higher than 15°C than males. Significant differences were also observed with respect to age by an increase of body coverage with age.

Further analysis shows that in the range of 7°C to 27°C, the frequency of young people being outdoors was almost constant, but at higher temperatures, it decreased significantly by approximately 5%/°C. In elderly people the decrease is of the same order, but already starts at 22°C.

Therefore, the highest UV radiant energy (irradiance over body area and over time) is received by young females and the lowest by elderly males. Differences by age and gender in received radiant energy become more pronounced as temperature increases.

Environmental and behavioral factors influencing childhood UVR exposure.

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Keywords: UVR exposure; Children; Sun protection

Exposure to solar ultraviolet radiation (UVR) during early childhood is a well-established risk factor for the development of skin cancer later in life. In a previous summertime study, we found that rural children (age 4–18) spent significantly more time outdoors and received higher UVR doses (0.9 SED, 4.7% of ambient UVR) than urban (0.3 SED, 2.1% of ambient UVR) and suburban children (0.4 SED, 2.8% of ambient UVR) on school or kindergarten days [1]. However, limited empirical data exist regarding the extent of UVR exposure among the youngest children aged 3–6.

A recent study aimed to quantify individual UVR exposure in suburban Danish kindergarten children during summer [2]. The aim was to compare two distinct educational settings: a traditional kindergarten with a standard playground (n = 17) and a forest kindergarten where children spend the entire day in a forested outdoor environment (n = 20). Personal UVR exposure, and clothing coverage was recorded at three intervals throughout the day: 09:00–11:00, 11:00–13:00, and 13:00–15:00. Data were collected on both a sunny and a cloudy day.

On the sunny day, children in the forest kindergarten received a mean UVR dose of 2.4 SED (0.8–3.6), corresponding to 7.6% of ambient UVR. In contrast, children in the traditional kindergarten received a significantly lower mean dose of 1.0 SED (0.4–1.8), equivalent to 3.4% of ambient UVR ($p < 0.0001$). On the cloudy day, forest kindergarten children again received the highest UVR exposure (mean: 1.3 SED; range: 0.6–2.1; 5.3% of ambient UVR) compared to those in the traditional setting (mean: 1.0 SED; range: 0.2–1.6; 4.3% of ambient UVR), although the difference did not reach statistical significance ($p = 0.056$). Notably, children in the forest kindergarten wore significantly more clothing than those in the traditional kindergarten.

Despite recent sun protection campaigns, children in kindergarten still receive high daily UVR dose. Children in forest kindergarten, who spend the entire day outside, had the highest UVR dose but also a greater protection from clothing coverage. These findings highlight the need for effective sun protection strategies during kindergarten hours to mitigate long-term health risks associated with early-life UVR exposure.

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Increasing solar UV radiation in Central Europe

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Keywords: Solar UV Radiation; UV exposure; Human Health

Increasing solar ultraviolet radiation (UVR) has the potential to adversely affect humans, life on Earth, and the environment, including materials, with implications for both public health and ecosystem sustainability. Excessive UV exposure increases the risk of acute and long-term skin and eye diseases. Knowledge of the actual ground-level solar UV radiation is essential for assessing these risks. Precise measurements of this radiation, as conducted among others by the German solar UV monitoring network, offer valuable insights into its intensity, composition, and temporal variations over both short and long periods.

In a comprehensive analysis, over one million UV data sets from the Central European cities Dortmund (51.5° N, 7.5° E, 130 m a.s.l.) and Uccle (50.8° N, 4.3° E, 100 m a.s.l.) were analyzed, and temporal trends were calculated [1]. For trend analysis, a novel model was used considering both autocorrelation and variance heterogeneity. A validated imputation method [2] ensured that data gaps did not affect the trend results. In addition to UV spectra, ground-level data on global radiation and sunshine duration, as well as satellite data on atmospheric ozone, were examined. This comprehensive approach allowed to identify correlations with the measured ground-level UVR data as well as potential causes for solar UVR trends.

Descriptive analysis shows the impact of the annual ozone cycle and low-ozone events (LOEs) on ground-level UV radiation. LOEs elevate UV Index values by 10-20% during summer and up to 50% in winter. Additionally, the descriptive analysis demonstrates that the correlation between the UV Index and daily erythemal UV dose values is strongly influenced by cloud cover. A comparison of data from Dortmund and Uccle offers important insights into the regional transferability of the locally collected data.

The trend analysis reveals a significant increase in monthly UVR from 1997 to 2022. When considering all trends and influencing parameters together, the increase can be attributed to changes in cloud cover. This conclusion is supported by satellite observations of decreased cloudiness over parts of Central Europe during the period [3].

The findings of this study are crucial for the development of radiation protection concepts and preventive strategies aimed at minimizing health risks in the context of climate change.

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UV radiation exposure of outdoor workers with respect to the Ultraviolet Protection Factor (UPF) of working garments

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Keywords: UV radiation exposure; Outdoor workers; UPF; Working garments

EXTENDED ABSTRACT

Aim: A Clothing is a main pillar in protecting outdoor workers from adverse effects of solar ultraviolet radiation (UVR). The goal of this study is to determine the effectiveness of the UVR protection afforded to workers by their workwear with respect to their UVR exposure.

Material and Methods: The UPF of 35 non-UPF-certified garments was measured, including shirts provided by construction companies, billed caps, and neck guards. Further, the personal UVR exposure of volunteers working at a construction site at Vienna International Airport was determined in early July using polysulphone films positioned just below the nape. The remaining radiant exposure through garments and the UPF required of an item to reduce the remaining daily radiant exposure to 1 SED were calculated.

Results: UPF among clothing items varied from UPF 5 to 431. With some exceptions, the UPF correlates with a garment's areal density, but not with colour. The personal exposure was 22 SED/day on average but varied between occupation areas. The highest measured daily exposure was 44 SED, corresponding to 90% of ambient UVR. Only about half the provided shirts and 65% of all items would reduce this exposure to 1SED/day or less.

Conclusion: Construction workers may be exposed to high UVR and thus require high-UPF clothing to reduce daily exposure below an acceptable threshold of 1 SED. To sufficiently reduce remaining radiant exposure, we recommend that clothing items must provide a UPF of 80+, sufficient coverage, must not contain leachable UVR-absorbers, and must support workers' wearing comfort.

Mitochondrial intercellular transfer in response to UV-induced stress

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Keywords: Mitochondrial transfer; UVB irradiation; mitochondria; Skin tissue engineering

Content: Ultraviolet radiations (UVR) are genotoxic environmental factors affecting both genomic[1] and mitochondrial DNA[2]. Exposure of skin cells to UVR triggers DNA damage response (DDR) in which mitochondria play a key role by providing the ATP required for DDR but also by initiating the apoptotic cascade signaling. Wang and Gerdes showed that UVC exposure triggers intercellular mitochondrial transfer (MT), which rescues cells from apoptosis at early stage[3]. However, no studies have shown the impact of physiological UVB exposure on MT and the consequences of this MT.

Our hypothesis was that MT, in response to UVB, is a protective mechanism against UV-induced damage. Our objectives were to: (1) Evaluate MT between dermal fibroblasts following UVB exposure and (2) Demonstrate MT using a 3D skin model.

Materials and Methods: To determine the influence of UVB on MT, co-cultures of human dermal fibroblasts (HDF) and mouse embryonic fibroblasts (MEF) were exposed or not to different doses of UVB. Human mitochondria were specifically stained to visualize MT from HDF to MEF. A similar strategy, but by UVB-irradiating only one of the two cultures (HDF or MEF), was used to investigate the uni or birectional MT. To establish the signal driving MT, MEF/HDF co-cultures were incubated in pre-conditioned media from unirradiated or UVB-irradiated HDF. Cytokine array was performed on the conditioned media to identify potential molecules responsible for the initiating MT signal. We generated tissue-engineered skin substitute[4] using fibroblasts expressing mitochondrial-specific red-fluorescent tag to evaluate the MT in skin model following or not UVB radiations.

Results: We found that UVB exposure increases mitochondrial transfer between fibroblasts. Mitochondrial transfer is unidirectional, occurring from unirradiated cells to UVB-irradiated ones. The signal of this unidirectional intercellular communication is triggered by secreted molecules coming from UVB-irradiated cells. In a 3D skin model, our preliminary results show that MT happens in human reconstructed dermis.

Conclusion: The intercellular mitochondrial transfer occurring in the skin following UVB stress is another key element in cellular response to solar genotoxic stress. Mitochondria provides another level of UVR-response by allowing communication between cells. This implication of mitochondria in cellular communication following UVR-induced stress needs further understanding and might be key to prevention and treatment of skin cancer and photo-aging.

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Dynamic Transcriptomic Responses of Human Dermal Stem Cells to Ultraviolet Radiation: Implications for Melanoma Origin

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Keywords: UV irradiation; dermal stem cells; melanoma, transcriptomics

Melanoma represents the most aggressive and lethal form of skin cancer, with ultraviolet (UV) radiation exposure serving as a primary etiological factor. The precise identity of the progenitor cell harboring the DNA damage necessary for melanoma initiation remains an area of active investigation. According to the traditional paradigm, melanoma originates from either transformed, mature epidermal melanocytes or melanocyte stem cells residing in the bulge region of hair follicles. However, an alternative hypothesis has recently gained traction, suggesting that extrafollicular melanocyte stem cells, specifically dermal stem cells (DSCs) located within the human dermis, may function as the cell of origin. These multipotent precursors, derived from the neural crest, are of particular interest due to their dermal localization, which subjects them to both UVA radiation, capable of penetrating deeper tissue layers, and UVB radiation, which predominantly affects the superficial dermis.

To elucidate the cellular and molecular response to UV stress, we performed bulk RNA sequencing on donor-matched DSCs and melanocytes after controlled single and multiple UVB exposures with samples collected at early (6 hours) and late (24 hours) time points. Our data reveal that DSCs display a markedly more dynamic transcriptomic response than melanocytes, with a 5- to 10-fold increase in differentially expressed genes following UVB exposure. Gene ontology (GO) analysis showed enrichment in pathways related to DNA repair, chromatin remodeling, and apoptosis, putatively linked to a heightened sensitivity and coordinated molecular response of DSCs to UV-induced damage.
Together these findings highlight DSCs as a highly UV-responsive cell population and a possible model for studying early events in UV-driven melanomagenesis. This work provides the first in-depth transcriptomic characterization of DSCs under UV stress and lays the groundwork for future epigenetic, transcriptomic, and proteomic studies aimed at unraveling the mechanisms of melanoma initiation and progression.

Effects and uptake of luminescent micro- and nanoplastics labelled with lanthanide-doped upconverting nanoparticles in small aquatic organisms

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Keywords: *cryomilling*; *microplastics*; *nanoplastics*; *upconverting nanoparticles* (*UCNPs*)

The increasing occurrence of micro- and nanoplastics (MNPs) in aquatic environments poses significant risks to living organisms due to their ability to penetrate biological membranes, resulting in enhanced bioavailability and translocation through the trophic chain [1-3]. The rise in mismanaged plastic waste exacerbates this issue, yet monitoring MNPs remains difficult as they evade conventional detection methods. This study presents an innovative strategy for visualizing MNPs in biological systems using lanthanide-doped upconverting nanoparticles (UCNPs). UCNPs convert longer-wavelength excitation light (e.g., near-infrared, NIR) into shorter wavelengths (e.g., visible light) and offer several advantages over traditional fluorescent probes, including sharp emission bands, large Stokes shifts, and resistance to photobleaching [4]. These unique properties, combined with the high penetration of NIR light, make them ideal for visualizing MNPs in biological matrices with negligible background interference.

The study focused on the effects and uptake of luminescent MNPs in microscopic aquatic organisms, specifically rotifers and algae. Luminescent MNPs were prepared by cryomilling bulk polystyrene (PS) labelled with $NaYF_4$:Yb^{3+}, Er^{3+} UCNPs (UC-PS). Cryomilling under constant liquid N_2 supply simulated the mechanical abrasion of plastic particles in natural environments, resulting in obtaining irregularly shaped luminescent PS micro- and nanoparticles (UC-PS) [5]. Their size and shape was determined via dynamic light scattering (DLS), transmission electron microscopy (TEM), and brightfield microscopy. The incorporation of UCNPs into the PS matrix was confirmed through spectroscopic measurements and fluorescence microscopy.

Fluorescence microscopy revealed that both algae and rotifers aggregated around clusters of UC-PS MNPs. Notably, the luminescent MNPs were found within the bodies of rotifers, providing direct evidence of plastic uptake by these organisms. This research not only showcases the application of UCNPs as powerful tools for monitoring plastic uptake and distribution in aquatic ecosystems but also highlights the potential ecological impacts of MNPs.

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This research was funded by the National Science Centre, Poland under grant no. 2022/45/B/ST5/00604.

Bifunctional natural antioxidants targeting mitochondria for UVA-induced photoprotection

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Exposure UVA radiation (320–400 nm) induces excessive production of reactive oxygen species (ROS) and promotes the release of labile iron (LI) in skin cells, which catalyses further ROS generation and amplifies oxidative photodamage [1]. Although synthetic iron chelators can mitigate LI-mediated damage, their prolonged use may lead to cytotoxicity due to excessive iron depletion. This study aimed to identify bifunctional natural compounds with potent antioxidant and moderate iron-chelating properties capable of protecting skin fibroblasts from UVA-induced damage, with a particular focus on preserving mitochondrial integrity. Caffeic acid and catechin were selected based on their antioxidant potential and structural suitability for iron chelation. Primary human skin fibroblasts (FEK4, FCP5, and FCP8) were pretreated overnight with the compounds or their iron complexes (40 and 100 µM), followed by UVA irradiation at doses up to 500 kJ/m². Cell viability was assessed using MTT assays and flow cytometry, while iron chelation was quantified using the fluorescent LI sensors Cytosense LI and Mitosense LI [2, 3]. Pretreatment with these compounds significantly reduced both cytosolic and mitochondrial LI pools, with this effect persisting post-irradiation and correlating with enhanced photoprotection compared to untreated controls. Notably, the cytoprotective effects were diminished upon iron complexation, underscoring the importance of iron chelation in their mechanism of action. Furthermore, both compounds effectively reduced intracellular and mitochondrial ROS levels following UVA exposure (up to 350 kJ/m²), preserved mitochondrial membrane potential, and prevented ATP depletion and necrotic cell death. The study also estimated the pFe values of the compounds using LI sensors and confirmed their antioxidant capacity via the DPPH assay. These findings underscore the potential of mitochondria-targeting natural compounds such as caffeic acid and catechin as promising bifunctional agents for inclusion in sunscreen and daily skincare formulations. Their ability to simultaneously neutralize ROS and chelate labile iron offers a dual line of defense against UVA-induced oxidative stress, particularly at the mitochondrial level—an emerging target in skin aging and photodamage. Incorporating such compounds into topical products could enhance cellular resilience, reduce long-term skin damage, and support healthier skin aging through a more comprehensive photoprotective strategy.

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Benzoporphyrin derivative-based nanoparticles for the targeted photodynamic therapy of ovarian cancer

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Keywords: Photodynamic therapy; folate receptor; verteporfin; ovarian cancer;

EXTENDED ABSTRACT

Ovarian cancer (OC) is the fifth most frequent cause of death in women, claiming the highest mortality rate among gynecological cancers. The standard of care for advanced-stage OC is surgical debulking and platinum-based chemotherapy, followed by maintenance treatment with PARP inhibitors and/or bevacizumab. However, majority face relapse, resulting in a 5-year survival rate of 30%. To mitigate treatment-associated toxicity while augmenting therapeutic outcomes, targeted therapies emerge as promising avenues. Folate receptor (FR) is overexpressed in 70% of primary and 80% of recurrent OCs. Additionally, its expression is retained after chemotherapy. 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. Benzoporphyrin derivative (BPD) 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.[1]

Leveraging BPD's potential and FR specificity, novel self-assembled nanoparticles have been developed for the targeted PDT of OC.[2] A novel BPD dimeric molecule (dBPD) was first developed to enhance efficacy of the self-assembly nanoparticles (dBPD-NPs) and the specific delivery through glutathione. dBPD-NPs exhibited increased photoactivity, singlet oxygen production, biocompatibility. Furthermore, upon functionalization with folic acid (FA), FA-dBPD-NPs shown enhanced uptake by FR-positive OC relative to dBPD-NPs and improved PDT efficiency (**Figure 1**).[2] These findings underscore the potential of our nanoformulation in selectively eradicating OC cells *via* PDT, offering improved biocompatibility, specificity, and therapeutic efficiency.

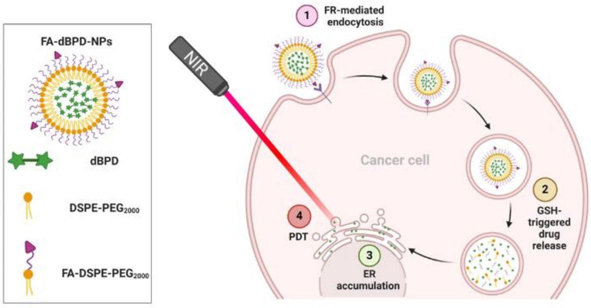

Figure 1. FA-dBPD-NPs components and intracellular therapeutic pathway.

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Targeted Nanoplatform to Overcome Delivery Barriers in Photoimmunotherapy and Chemotherapy for Peritoneal Cancer

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Keywords: peritoneal metastasis, nanodrug, photoimmunotherapy

Abstract

Peritoneal metastasis affects over one million individuals annually, often with devastating outcomes [1]. It arises from the direct shedding of tumor cells that are commonly from ovarian, colorectal, and other abdominal cancers. These cells seed and grow along the peritoneal surfaces and internal organs. While standard chemotherapy and surgery can offer temporary symptom relief, durable disease control and prolonged survival remain rare due to residual tumor burden and treatment resistance. Over the past four decades, significant progress has been made in the development of photoimmunotherapy (PIT) for treating metastatic disease. However, clinical translation remains challenging, largely due to variability in photosensitizer uptake and heterogeneous treatment response. Our prior work has demonstrated that integrating fluorescence-guided intervention, targeted nanomedicine, and a cloud-connected medical laser system (ML7710) can enhance both the consistency and effectiveness of photodynamic therapy for managing micrometastasis [2,3]. In this presentation, we will introduce a next generation, actively loaded nanodrug formulation. We will share new findings on tumor penetration depth and therapeutic efficacy which highlights the potential to advance the clinical translation of PIT for treating peritoneal metastasis.

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Photodynamic drug delivery: Designing liposomes for light-controlled release and enhanced chemotherapy efficacy

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Keywords: Drug delivery; Cancer; Photodynamics; Liposomes.

Liposomes have been the most successful type of nanomedicine for the treatment of cancer, capable of carrying chemotherapeutics with higher precision towards cancer tissues [1]. While liposomes can improve chemotherapy safety, the treatment efficacies are rarely improved due to a lack of spatioemporal control of drug release and the poor penetration of drugs into the dense cancer microenvironment. Within the field of drug delivery, photodynamics has emerged as a strikingly effective approach for spatiotemporal-controlled drug release and improved drug efficacies by harnessing photochemical redox reactions to destabilize lipid nanoformulations that contain oxidation-susceptible excipients [2].

This study outlines key design considerations for lipid nanoformulations in (1) light-controlled drug delivery, (2) overcoming lysosomal drug sequestration, and (3) facilitating microenvironmental priming to enhance tumor permeability [3]. These considerations first highlight the role of specific lipid excipients in determining photodynamic drug release efficiencies. Secondly, the selection of the photosensitizing agents is considered, which ideally absorb light >650nm and exhibit limited leaching. Thirdly, the selected photosensitizing agent and pharmaceutical cargoes may dictate which drug loading approach should be pursued, and how drug release is detected. Finally, considering the corollary effects of photodynamics on cancer cells and the cancer microenvironment, we emphasize the utility of a multi-model approach to evaluate novel photodynamic drug delivery systems.

These design considerations are intended to boost the field of photodynamic drug delivery and encourage its exploitation in translational cancer research. Moreover, these aspects are equally relevant to stimulate investigations towards oxidation-responsive drug release triggered by alternative external stimuli such as X-rays [4], thereby broadening the drug delivery arsenal against cancer.

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Investigating photodynamic priming-radiotherapy synergy: A mechanistic insight into the efficacy of nanoscintillator-mediated PDT

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Keywords: Photodynamic therapy, Photodynamic priming, Radiotherapy, Nanoscintillators

Photodynamic therapy (PDT) is limited by the shallow tissue penetration of visible light, making it unsuitable for deep-seated tumors without invasive techniques [1]. A non-invasive solution involves conjugating photosensitizers to radioluminescent nanomaterials (nanoscintillators), which emit UV or visible light upon X-ray exposure, to locally activate PDT [2]. Since X-rays penetrate deeply in tissues, this strategy enables PDT activation at depth, overcoming traditional light delivery constraints and expanding treatment to larger tumor volumes. The feasibility of nanoscintillator-mediated excitation of photosensitizers has been demonstrated by our group and others [3-5]. However, a notable discrepancy exists between the relatively low theoretical efficacy predicted using physical models for radioluminescence-induced PDT and the substantially higher efficacy observed experimentally. This suggests the involvement of additional (biological) mechanisms that may have been underappreciated or previously overlooked. One such mechanism is the enhanced radiation dose deposition mediated by the high atomic number (Z) elements commonly found in nanoscintillators, which may amplify local radiation effects [6], [7]. Another key area of investigation is the potential synergistic interaction between PDT and radiation therapy, which are mechanistically distinct but may act cooperatively when combined. Importantly, the scintillation yield of nanoscintillators is significantly lower than the irradiance produced by conventional laser sources. While the close proximity of emitted light to the photosensitizer mitigates tissue attenuation and scattering, the overall light dose may still fall within the photodynamic priming (PDP) range. PDP refers to sub-lethal PDT regimens that modulate the tumor microenvironment and may sensitize cells to subsequent therapies, including radiation.

This presentation will explore the combination of PDP with radiotherapy, focusing on their potential synergistic effects. We propose that harnessing this synergy could enhance the efficacy of nanoscintillator-induced PDT and represent a powerful advancement in non-invasive cancer therapy.

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Overcoming Chemoresistance in Cancer: A Mechanistic Look at PDT and ABC Transporters

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Keywords: Photodynamic therapy; drug resistance; cancer;

Drug resistance is a complex and multifaceted problem, well-documented in microbiology where it evolves rapidly. In cancer therapy, drug resistance poses a similarly formidable challenge, sharing many features with microbial resistance. The term multidrug resistance (MDR) is used in both contexts to describe the ability of cells to evade the effects of multiple therapeutic agents through various mechanisms. A major contributor to MDR is the family of ATP-binding cassette (ABC) transporters, which actively pump drugs out of cells or sequester them, preventing therapeutic action at their intended targets. Additional resistance mechanisms include the overexpression of anti-apoptotic proteins and enhanced DNA repair pathways. This presentation highlights how photodynamic therapy (photochemistry) can be strategically employed to overcome ABC transporter-mediated MDR [1]. Through a combination of in silico, in vitro, and in vivo studies, we demonstrate that porphyrin-based photochemistry selectively impairs ABC transporters overexpressed in chemoresistant cancer cells via three distinct mechanisms [2,3]: (i) Inhibition of ATPase activity, disrupting the energy source required for drug efflux; (ii) Induction of protein crosslinking, potentially destabilizing transporter function; (iii) Suppression of transporter expression and function, reducing the cell's drug-pumping capacity. Our findings support porphyrin photochemistry as a promising and targeted approach to overcoming MDR in cancer treatment.

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Localization of laser thermal therapy in gliomas using plasmonic gold nanorods

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Keywords: gold nanorods; glioblastoma; photothermal therapy; fibroblast growth factor-inducible 14 (Fn14) receptor

Glioblastoma (GBM) is an extremely aggressive, invasive type of brain tumor with a 1-year survival rate of ~30% with 5% surviving 5 years [1]. With over 300,000 new diagnoses worldwide annually, and a median overall survival of ~14 months, there have been no notable improvements in patient outcome in several decades [1,2]. MRI-guided laser interstitial thermal therapy (LITT) is a promising new minimally invasive treatment that relies on endogenous chromophores, like water and hemoglobin, to generate heat from high power laser light (12-15 W). However, this generates non-specific heating leading to damage of healthy tissue and side effects [3,4]. Gold nanorods (GNRs) are efficient photothermal agents due the surface plasmon resonance effect, allowing, in combination with LITT, the laser power to be lowered and heat localized to areas including GNRs [5]. Fibroblast growth factor-inducible14 (Fn14) is a cell-surface receptor for TNF-like weak inducer of apoptosis (TWEAK) and is overexpressed in 85% of GBM tumors [6]. We engineered decreased adhesivity, receptor-targeted GNRs (DART-GNRs) decorated with polyethylene glycol and anti-Fn14 antibodies for GBM selectivity [7].

DART-GNRs are excitable in the NIR range and show an improved rate of heating compared to endogenous chromophores. In brain-mimicking agarose phantom models, DART-GNRs have demonstrated localized heating by achieving therapeutic temperatures near the laser fiber while maintaining safe boundary temperatures. To represent the invasive and infiltrating cells characteristic of GBM, different shapes of GNR areas were used in phantom models and confirmed that only GNR-containing areas are heated at low laser powers (<2 W) to therapeutic temperatures. The ability to generate photothermal effect in vivo was confirmed using a human GBM39 PDX intracranial mouse model. We demonstrated the CT imageability of GNRs in phantom models and mouse brains along with the ability to quantify the attenuation (Hounsfield units) to improve safety of LITT treatment by enabling pre- and post-operative tracking. DART-GNRs show enhanced photothermal killing and accumulation in Fn14-positive GBM cells compared to non-targeted GNRs and GBM cells lacking Fn14. These results suggest that DART-GNRs are a clinically viable photothermal agent that may improve current LITT treatments for GBM patients.

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N-Acetylcysteine as a co-adjuvant in photodynamic therapy for the treatment of cutaneous squamous cell carcinoma

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CONTEXT

Although photodynamic therapy (PDT) is distinguished by its high efficacy and minimal cosmetic impact, resistance remains a significant challenge. The relapse rate for PDT five years post-treatment ranges from 18% to 50%, with up to 50% of cases exhibiting resistance. Numerous studies have identified transforming growth factor β1 (TGFβ1) secreted by cancer-associated fibroblasts as the potential contributor to therapy resistance across various cancer types. In this line of work, the combination of PDT with agents that amplify its efficacy and mitigate resistance remains a critical objective. In this context, drug repurposing presents a promising approach. Previous studies have demonstrated that N-acetylcysteine (NAC), a drug approved for the treatment of pulmonary fibrosis, significantly reduces TGFβ1 levels in human fibroblasts culture. This effect of NAC on TGFβ1 signaling is particularly promising to favor cancer therapy. The potential of this compound to target complex diseases linked to TGFβ1 motivates its use in combination with PDT, with the aim of enhancing treatment efficacy by mitigating tumor resistance

HYPOTHESIS

Our study proposes NAC as a co-adjuvant to enhance the efficacy of PDT. Consequently, the combination of NAC and PDT may improve the treatment of cutaneous squamous cell carcinoma (cSCC) by counteracting the effects of cancer-associated fibroblast-derived TGFβ1.

METHODOLOGY

To evaluate this approach, we investigated the effects of PDT combined with NAC in both *in vitro* and *in vivo* models. For the *in vitro* analysis, we utilized a spheroid model derived from cSCC. For the *in vivo* study, SKH-1 mice were chronically exposed to ultraviolet light to induce cSCC. Then, NAC was topically applied to the SKH-1 mice prior to MAL-PDT treatment.

RESULTS AND CONCLUSION

Our findings demonstrated that NAC effectively prevented resistance in cSCC spheroids. Furthermore, the combination of PDT and NAC led to an enhanced therapeutic outcome in the SKH-1 mouse model, highlighting this approach as a promising strategy to overcome PDT resistance.

MISSION TO KILL – INVESTIGATING NOVEL PHOTOCHEMICAL STRATEGIES TO ELIMINATE CHOLANGIOCARCINOMA

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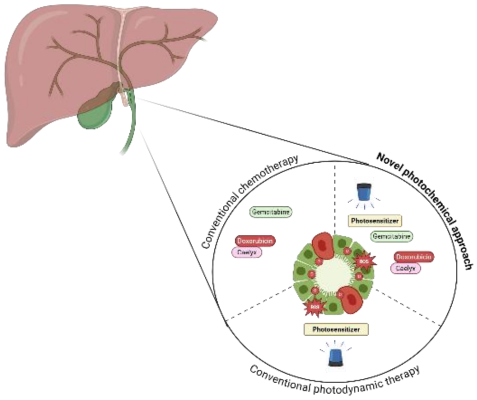
Keywords: Photochemical Treatment, Chemotherapy, Cholangiocarcinoma, mRNA Sequencing, Spheroids



Cholangiocarcinoma (CCA), commonly known as bile duct cancer, is a rare but extremely lethal epithelial cell malignancy that occurs anywhere along the biliary tree and/or within the hepatic parenchyma. Early stage CCA is asymptomatic, and most cases are diagnosed at an advanced tumor stage with poor prognosis and limited treatment options (Banales et al., [2016]; Valle et al., [2017]). Standard first-line palliative treatment involves systemic chemotherapy with gemcitabine and cisplatin. However, due to the tumor's high resistance against chemo- and radiation therapy, and the lack of efficient drug delivery methods, long-term treatment outcomes remain poor (Banales et al., [2016]).

To address this issue, we explore the potential of photochemical treatment (PCT), an approach that aims to improve treatment response by combining cytotoxic drugs with photodynamic therapy (PDT) (Berg et al., [2011]). After we examined the uptake dynamics and confirmed the cytosolic release of the endosome-targeting photosensitizer, TPCS2a (*fimaporfin)* in CCA cell lines we investigated the efficacy of our approach, i.e. combined PDT and Gemcitabine treatment.

With short- and long-term cell viability assays, we show that PCT is more efficient than conventional chemotherapy or PDT alone. Further, we used mRNA sequencing to map early and late transcriptomic changes in CCA cells after Gemcitabine, PDT and photochemical treatment. The most striking results included a pronounced upregulation of chaperone/heat shock proteins (HSPs), the activation of the MAPK signaling cascade, and increased expression of genes regulating the PD-1/PD-L1 axis, apoptosis, autophagy and ferroptosis. To follow up on these results, we first examined apoptosis by tracking caspase 3/7 activity with the *IncuCyte® Live-Cell Analysis System*. Next, we assessed immunogenic cell death by measuring ATP, HMGB1 and HSP90 secretion after treatment. Our results are consistent with the transcriptomic data: The levels of ATP, HMGB1 and HSP90, as well as the caspase 3/7 activity, were higher after PCT compared to conventional therapy. Furthermore, preliminary data suggests an enormous autophagic capacity in the EGI-1 cell line.

The ongoing work involves targeted inhibition of the identified pathways as well as verification studies in EGI-1 spheroids and patient-derived primary sclerosing cholangitis organoids.

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Light-induced disruptive porphysomes with improved photodynamic applications against cancer and bacterial infections

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Keywords: Porphysome, photodynamic therapy, photothermal therapy, porphyrin

Porphysomes[1] are a class of light-responsive liposome-like nanoparticles, which result from the supramolecular assemblies of phospholipid-porphyrin (PL-Por) conjugates. They have proven their efficacy in photothermal therapy (PTT) but less in photodynamic therapy (PDT) against cancer or bacterial infection because of the intensive fluorescence quenching in the assemblies.[1-2] Herein, we present the design of a novel generation of smart porphysomes capable of undergoing active dissociation and releasing porphyrin moieties upon illumination, thereby enabling tunable photothermal properties with enhanced photodynamic efficiency. The new porphysomes are composed of intelligent PL-Por conjugates that exhibit one or two ROS-responsive linkers separating the polar head group from the porphyrin moiety. Among the designed molecules, the monosubstituted conjugates bearing either Pyro-a or Pheo-a porphyrinoids showed the best performance in terms of stability, photothermal and photodynamic efficiencies *in vitro*. Moreover, these assemblies were found to achieve complete tumor ablation in 80% of PC3 prostate subcutaneous tumor-bearing mice after 30 days post-PDT, compared to 0% using conventional porphysomes.[3] Besides cancer therapy, we applied the new conjugates against Gram + and Gram – bacteria as well as against their biofilm, the new porphysomes eradicate the bacteria with an improvement up to 10 folds in their antibacterial efficiency against bacteria and biofilm. Consequently, our strategy enabled the development of a versatile platform for delivering porphyrin-based photosensitizers for enhanced photodynamic applications.

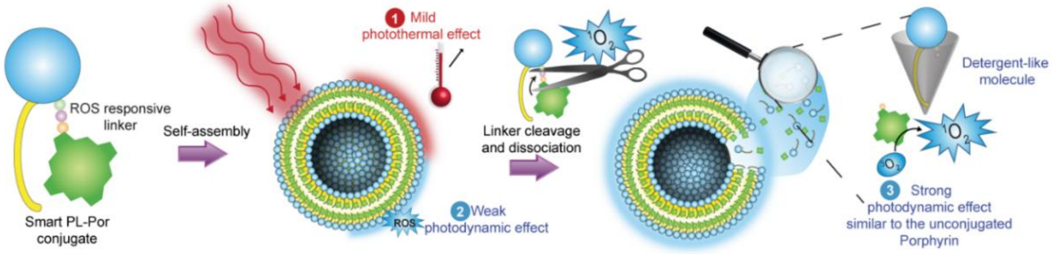

Figure 1. Scheme representing the concept of the new porphysomes generation.

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Harnessing Dynamic Supramolecular Polymers for Targeted Photodynamic Therapy

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Keywords: Supramolecular polymers; Photodynamic therapy; Photosensitisers.

Photodynamic therapy (PDT) has become a clinically promising approach for the treatment of a wide range of cancers and other diseases due to its spatiotemporal selectivity and non-invasive nature. However, the use of traditional molecular PSs has inherent drawbacks that hamper the full development of PDT, as the combination of poor transport, low tumor selectivity and the "always-on" property generally leads to post-treatment risks associated with harmful photosensitization.

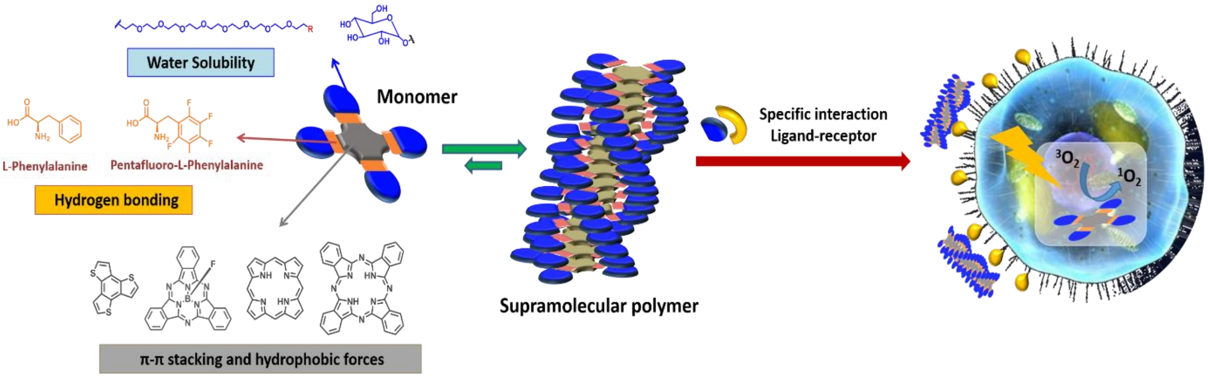

Figure 1. Schematic representation of the monomers, their self-assembly and applications.

The lecture will discuss new strategies on the design, synthesis and self-assembly study of different monomeric photosensitizers in aqueous media [1], [2], and the advantages when employing the resulting supramolecular assemblies in PDT or photothermal therapy. [3], [4]

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Nanostructured amphiphilic phthalocyanines and metallo-supramolecular porphyrinoid assemblies for targeted phototherapy

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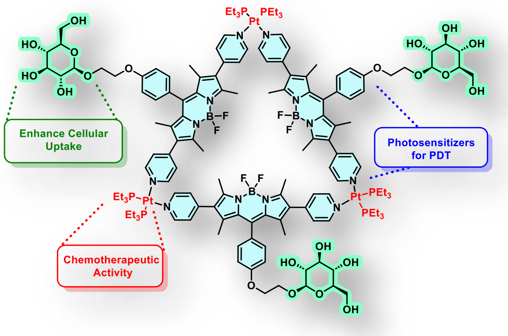
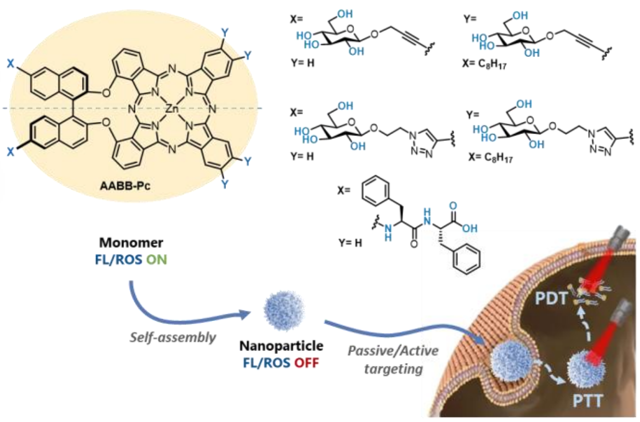
Keywords: Metallacycles; Phthalocyanines; Self-Assembly; Nanoparticles

Nanoparticles (NPs) represent an emerging technology in the field of Photodynamic Therapy (PDT) that can overcome most of the limitations of classic photosensitizers (PS). Particularly relevant is the development of nanosystems through the self-assembly of customized PS, providing improved transport properties and selective uptake by target cells. To achieve self-assembled NPs with high phototoxicity towards cancer cells, we have followed two approaches. First, we have prepared Pt(II)-BODIPY complexes that combine the chemotherapeutic activity of Pt(II) with the PDT effect of BODIPYs, which is enhanced by favored singlet-to triplet intersystem crossing due to the coordination to the heavy metal [1]. Also, conjugation with glucose targeting ligands plays a two-fold role: i) increases the amphiphilicity of the metallacycle, boosting its self-organization into NPs; and ii) enhances the uptake by cancer cells that overexpress the corresponding receptors. On the other hand, we have prepared a series of strongly amphiphilic Zn(II)phthalocyanines (Zn(II)Pcs) designed to self-assemble into stable NPs in aqueous media, and evaluate them as nanoagents for PDT, antimicrobial-PDT, and/or Photothermal Therapy (PTT) [2]. We envisage the preparation of Zn(II)Pc scaffolds with an AABB substitution pattern, following the synthetic methodology previously developed in the group [3], which incorporate different hydrophilic substituents and/or biological vectors that direct their accumulation into specific biological targets.





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Cluster and kill: the use of clustering-triggered emission materials for antimicrobial photodynamic therapy

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Keywords: Cluster Triggered Emission, Aggregated Induced Emission, Through Space Interaction, Antimicrobial Photodynamic Therapy, Singlet Oxygen.

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.[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-lived excited states 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*).[3] 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.

Funding: This work was supported by funds from the Spanish Ministerio de Ciencia e Innovación-Agencia Estatal de Investigación (MCI/AEI/10.13039/501100011033) and FEDER (Projects PID2020-115801RB-C22 and PID2023-149483NB-C22). S.N. thanks the Departament de Recerca i Universitats de la Generalitat de Catalunya for the support given to our research group (2021 SGR 01023) and the ICREA-Catalan Institution for Research and Advanced Studies for grant No. Ac2232308. R.B.O. thanks the Spanish Agencia Estatal de Investigación for a Ramon y Cajal contract (RYC2021-032773-I)

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Modular nanoparticles for bioimaging and photodynamic therapy of breast cancer

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Keywords: cancer; metal organic frameworks; bioimaging; photodynamic therapy;

Photodynamic therapy (PDT) is nowadays improving by developments in nanotechnology. There are many possibilities to create functional materials. Great attention is given to targeted drug delivery that can be focused on micro- and mesoporous nanoparticles [1, 2], especially metal-organic frameworks (MOFs) [3].

Results: UiO-66 porous material used in this study is very attractive in PDT due to its fluorescence properties. This material is based on zirconium (IV) ions forming hexanuclear clusters $[Zr_6O_6(OH)_6]^{6-}$ bridged by a derivative of terephthalic acid containing amine functional group and is characterised by an extensive surface area, a large pore volume and a high thermal stability (up to 500 °C). It shows *pH*-dependent stability and is resistant to degradation under extracellular conditions and disintegration in intracellular environments. UiO-66 loaded with methylene blue were taken by breast cancer cells and their irradiation resulted in decreased viability. The combination of PDT with chemotherapy significantly improved the treatment.

Conclusion: The prepared modular nanocarriers presented high biocompatibility and minimal dark cytotoxicity *in vitro* and are well tolerated *in vivo*. The multimodality of the nanoparticles enables the identification of transport system, photosensitizer and active molecules in cells and tissues. Overall, the developed system presents promising properties for photodynamic therapy of cancer and the photo-inactivation of bacteria.

Acknowledgement: This research was funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under project No. 09I03-03-V04-00007.

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Smart Photoactivatable Assemblies for Eradicating Local Antibiotic-resistant Bacterial Infections

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Keywords: photoactivatable assemblies, photodynamic therapy, photothermal therapy, targeting bacteria

Nowadays, the world is running out of efficient antibiotics that can eradicate antibiotic-resistant bacteria. According to WHO, in the absence of new treatments to combat rising antimicrobial resistance, the death rate will drastically increase from 700,000 to 10 million per year by 2050 [3]. Therefore, usage of phototherapeutics such as photodynamic therapy (PDT) that relies on the combination of 3 components: photosensitizer, light, and oxygen to generate reactive oxygen species (ROS), and photothermal therapy (PTT) that requires the use of photothermal agent and light to produce heat, has emerged [1,2].

In this research, the combination of PDT and PTT without any usage of antibiotics has been proposed for the efficient obliteration of different types of bacteria, such as *Staphylococcus aureus, Corynebacterium glutamicum,* and *Pseudomonas aeruginosa,* which possess different membrane structures. Thus, photoactivatable assemblies known as “Porphysomes” were formulated using smart lipid-porphyrin conjugates exhibiting high fluorescence quenching for effective PTT. Moreover, the latter includes integration of a ROS-responsive linker that leads to controlled release of a photosensitizer for enhanced PDT effect. This strategy might decrease collateral damage to healthy tissues, which is usually characteristic of conventional photothermal agents due to overheating. Several lipid-porphyrin conjugates were synthesized, which were used for assembly formation. Some of the assemblies were composed of targeting moieties to target specific overexpressed receptors on the surface of bacteria or biofilm. Formulations demonstrated a mild photothermal effect in suspension, ranging from 14-21 °C, with higher photodynamic activity compared to conventional porphysomes. Following the characterization, *in vitro* tests were conducted on planktonic bacteria by illuminating the treated bacterial suspension for 10 minutes and counting the surviving bacteria by the colony-forming unit (CFU) method. *Staphylococcus aureus* was obliterated with 0.01 µM concentration of the photosensitizer, whereas 1 µM was enough to completely eradicate *Corynebacterium glutamicum,* and lastly, 10 µM for *Pseudomonas aeruginosa*. The concentration ranges due to their membrane structural complexity and sensitivity to PDT.

To conclude, *in vitro* results demonstrated efficient eradication of several bacterial strains using photoactivatable assemblies with PDT/PTT capabilities. Consequently, this study marks notable progress in the development of therapies against antibiotic-resistant bacterial infections.

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Synthesis and Self-Assembly of Glycosylated Aza-BODIPY Derivatives for Bioimaging

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Keywords: Self-assembly; aza-BODIPY; near-infrared light; photoacoustic

The use of self-assembled photoactive molecules in nanomedicine is gaining attention nowadays for their promising potential in specific bioimaging and phototherapy applications. Supramolecular chemistry provides fabrication strategies that have evolved from the creation of thermodynamically stable structures to kinetically trapped and far-from-equilibrium architectures, and more recently, to dynamic, adaptive materials with spatiotemporally responsive features. Mainly, these systems have been studied in several areas of nanomedicine, such as, bioimaging (fluorescence imaging and photoacoustic imaging) and biosensing, drug delivery, phototheranostic treatments (photodynamic and photothermal therapy) among others. [1,2]

In this context, aza-BODIPYs are excellent candidates as they have strong absorption and emission in the near-infrared (NIR) region (within the optical window for biological studies), which can be finely tailored through molecular design and through supramolecular aggregation.[3]

In this work, we have synthesized two amphiphilic aza-BODIPY structures (Figure 1) bearing different carbohydrate substituents, which are expected to impact their selective accumulation into cancerous cells, as well as their self-assembly abilities in aqueous media. The monomeric forms show good absorption in the NIR due to a PET process from the dimethylaminophenyl units, which can be red-shifted following a controlled aggregation process (930-959 nm) in aqueous media. Consequently, the new supramolecular species have proved excellent photoacoustic properties in *in vitro* studies on cancerous cells.

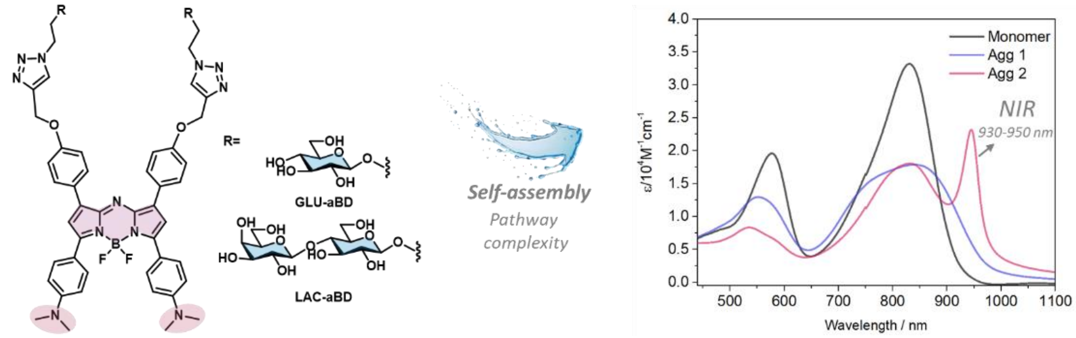

Figure 7. Structure of the glycosylated aza-BODIPYs (left) and the UV-Vis spectra of the new supramolecular species formed in aqueous media (right).

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Phthalocyanine-Encapsulated Lignin Nanoparticles for Photodynamic Applications: Data Analysis Assisted Nanoengineering

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Keywords: Lignin nanoparticles; Nanoencapsulation; Photodynamic Therapy; Phthalocyanine

Lignin, an abundant biopolymer and by-product of the paper industry, has gained increasing interest in biomedical research due to its low cytotoxicity and biodegradability [1]. Phthalocyanines (Pcs), well-known for their strong absorption in the phototherapeutic window, are effective photosensitizers for photodynamic therapy. However, their tendency to aggregate in aqueous environments due to their hydrophobic nature limits their clinical performance [2]. Encapsulation into lignin nanoparticles is considered as a promising solution to enhance their dispersion, stability, and photodynamic activity.

Three Zn(II) Pcs with different solubility profiles were selected and encapsulated into acetylated and methylated lignin nanoparticles via a dialysis-based method. Various formulation parameters—including Pc structure, lignin type, solvent used for solubilization, Pc-to-lignin ratio, and dialysis time—were systematically investigated. The resulting nanoparticles were analyzed using UV-Vis spectroscopy, dynamic light scattering (DLS), and electron paramagnetic resonance (EPR). To complement the experimental findings, a data-driven approach was also applied to identify the most critical factors influencing nanoparticle characteristics.

Detailed insights into the nanoparticle formulation process and how specific experimental parameters impact the structural and functional properties of the final nanoparticles will be discussed.

Acknowledgments

This work was founded by French Ambassy in Türkiye via Mobility Fellowship for PhD Students and by Acıbadem University APAPKO research funding (Biopholina project 2022/02-06). The author also gratefully acknowledges support from TÜBİTAK's BİDEB 2211-E National PhD Scholarship Program for Former Undergraduate and MSc/MA Scholars.

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Don't Help Them to Bury the Light. Multiscale Methods to Simulate Photo-Biological Phenomena

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Keywords: Molecular Modeling; Non-adiabatic Dynamics; MD Simulations; Photoimmunotherapy.

The interaction of biological systems with external stimuli, such as light, is responsible for crucial phenomena related to signaling as well as energy conversion, and storage. Furthermore, while external perturbations, and thus light, may induce harmful effects related most notably to skin cancer development, they can be exploited for therapeutic purposes, for instances in Photodynamic or Light Assisted Therapy approaches. In this talk, we will illustrate the crucial role played by molecular modeling and simulation in elucidating these complex processes and their interaction patterns in biological systems. We will stress the necessity of describing multiscale phenomena considering at the same time the intricate electronic structure rearrangements and its interplay with dynamic sampling and time evolution in biological macrostructures. By using appropriate and high-level original molecular modeling and simulation strategies we will prove that the scientific field is nowadays ready to provide answers to, and hence rationalize, biological questions related most notably to DNA mutation and repair and cell signaling. We will also see how molecular modeling can assist in developing original photoimmunetherapy approaches. Thus, we will show how molecular modeling can describe photobiological phenomena from the fast light matter interaction up to slower the biological outcome. We will prove that we are clearly leaving the age of the simulation of model systems to enter the age of a veritable *in silico* photobiology.

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Multiscale Simulation of a Fluorescent Probe Sensitive to Different DNA Structural Conformations

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Keywords: DNA; Fluorescence probe; Molecular dynamics; Computational chemistry

Guanine quadruplex (G4) is considered one of the most relevant DNA non-canonical structures, as alternative to the canonical double stranded (ds) B-DNA. G4s are well known for their role in gene expression and cancer, as they are found in many oncogenic promoters, both at the ends of DNA sequences (telomeres) or elsewhere [1]. To detect the potential zones of the DNA sequence where G4s are formed in living cells, near-infrared fluorescent probes were proposed. Here, we explain our multiscale simulation approach (molecular docking, molecular dynamics, quantum mechanics and hybrid quantum mechanics/molecular mechanics) of the tripodal quinone-cyanine dye QCy(MeBT)$_3$, featuring a star-shape able to stably interact with both B-DNA and G4 conformations. Indeed, it was the first experimentally reported switch-on fluorescent probe able to distinguish between B-DNA and G4 through different fluorescence colors [2]. Our results match the experimental outcome, also considering analog probes [3]. Hence, our simulation shines light on largely unclear aspects of the probe/DNA binding and selective fluorescence properties, with the aim of designing more selective DNA probes and understanding how to stabilize G4 conformations.

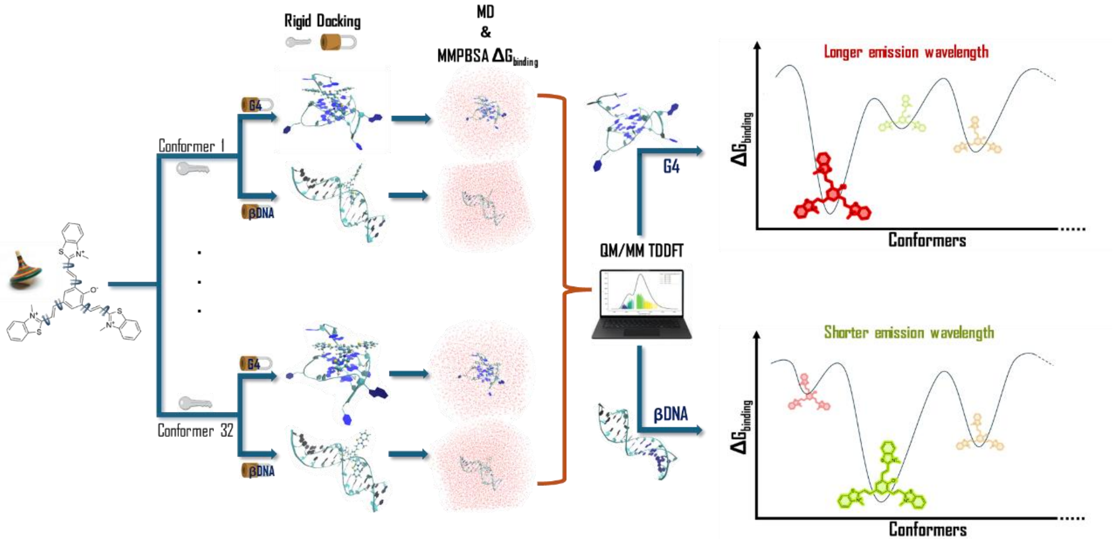

Figure. Multiscaling approach to obtain simulated fluorescence spectra of QCy(MeBT)$_3$ in interaction with G4 and B-DNA (Credit: Lorenzo Gramolini).

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Leveraging computational methods to characterize the photophysical features of emerging light-activated anticancer agents

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Keywords: PDT; DFT/TDDFT; Light-driven Therapies; Anticancer

Offering spatio-temporal control over drug activation and reduced side effects compared to conventional treatments, photodynamic therapy (PDT) stands out as the most advanced approach among the wide range of photoactivated anticancer strategies. In this field, computational approaches play a pivotal role in guiding the rational design and the understanding of next-generation photosensitizers with enhanced performance in oxygen-deprived environments. Actually, the limited availability of molecular oxygen in many tumor microenvironments is one of the main factors limiting the clinical efficacy of the treatment. Addressing this challenge requires the development of novel light-responsive agents capable of operating effectively under hypoxic conditions, as well as the exploration of alternative therapeutic mechanisms that do not directly rely on oxygen.

This contribution highlights recent insights derived from DFT/(QR)TDDFT and multiscale computational methods, which have been crucial in identifying the distinctive features of metal-based and metal-free systems with unprecedented hypoxia activity, as revealed in our combined experimental-computational investigations [1-3]. Additionally, it presents the exploration of photosensitizer's biotinylation effects as a novel strategy to enhance ROS production efficiency [4].

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Aknowlegments
PRIN D.D. 104/2022 PNRR M4.C2.1.1. – Project Code: 2022AN47CA – CUP: H53D23003820001, funded by the European Union – NextGenerationEU – Project Title "LA-G4-DACA" – Grant Assignment DD 862, 16/06/2023 is aknowledged.

The photophysics of thieno and isothiazolo guanine fluorescent analogues: insights from quantum mechanical calculations

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Keywords: One; Two; Three; Four; (up to 4 words)

Nucleic Acids suffer from the very low fluorescence quantum yield of their natural nucleosides, explaining the efforts to develop fluorescent nucleoside analogues (FNAs) to report locally on DNA structure and interactions. FNAs should ideally (i) replace natural nucleosides without any modification of DNA structure and function, (ii) keep high brightness, (iii) exhibit spectroscopic properties sensitive to the environment and ultimately (iv) reveal quantitative, local information on DNA structure and its interactions with biomolecules. An important advance has been recently achieved with the development of the thieno-guanosine analogues thG and ^{tz}G (Fig. 1), which perfectly replace G in DNA duplexes, being truly isomorphic, while keeping high quantum yields environmental sensitivity.[1-7] In order to validate thG and ^{tz}G as fluorescent mimic of guanosine, it is necessary to associate any change in their photophysical properties with a well-defined electronic structure and conformation of the probe and its nearest interacting neighbors. In this contribution we shall discuss the role of Quantum Mechanical calculations to achieve such a picture, based on calculations on systems of different size and complexity, from the isolated FNA in solution, to double and quadruple helices.

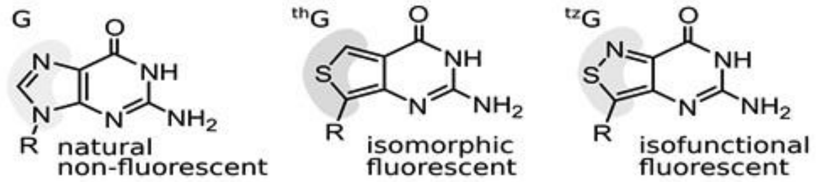

Figure 1. Structures of deoxyguanosine (G), deoxythienoguanosine (thG) and deoxyisothiazologuanosine (tzG). R = 2'-deoxyribose.

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Acknowledgements: RI, thanks the CNR program “Progetti di Ricerca @cnr", project UCATG4, N and CN3, National Center for GeneTherapy and Drugs based on RNA technology, funded by the European Union-NextGenerationEU-PNRR for financial support.

QM/MM-MD investigations of DNA probes and photoDNAzymes: towards molecular design

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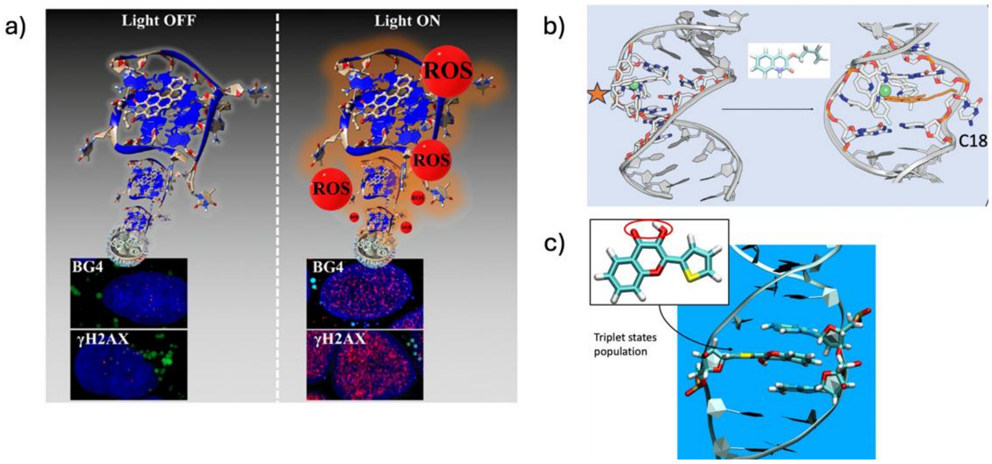
Keywords: photoDNAzyme, triplet-triplet energy transfer, molecular dynamics, QM/MM

EXTENDED ABSTRACT

DNA offers a unique and inspiring playground for chemistry, yet the photophysics and photochemistry of engineered luminescent DNA probes, photoactivable G-quadruplexes ligands or photocatalysts can be significantly tuned by the environment. In such contexts, one can increasingly rely on molecular modeling techniques to corroborate conformational hypothesis and generate mechanistic pathways.

Molecular simulations of damaged or chemically-modified oligonucleotides or photoactivatable DNA-ligand systems enable to generate molecular structures that allow to delineate most favorable pathways. I will illustrate several tailored strategies on several systems in which classical (enhanced) MD, QM/MM(-MD) and non-adiabatic molecular dynamics simulations have been deployed to either a) unravel DNA-ligand binding modes, b) elucidate the structure and mechanism of a photoDNAzyme [3] and c) capture the active hydration role of a DNA fluorescent probe and quantify its triplet states population [2].



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Copper Nanoclusters as Vector to Cure Menkes Disease: Insights from Molecular Modeling

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Keywords: Copper Nanoclusters; Menkes Disease; Molecular Modeling

Menkes disease is an extremely rare genetic disorder affecting copper metabolism, with an average life expectancy of around three years [1]. It is caused by a deficiency in a copper transporter found in both the intestine and the blood-brain barrier, leading to severe copper depletion and a subsequent shortage of essential cuproproteins.

Recent experimental studies have suggested that administering small copper nanoclusters stabilized in solution by selected amino acids may offer a promising strategy to mitigate copper deficiency in Moblo mice models [2]. However, understanding the synthesis, characterization and behavior of these novel copper delivery systems remains challenging.

In this presentation, we will demonstrate how a comprehensive molecular modeling approach, combining electronic structure methods with classical molecular dynamics, can complement UV/vis absorption and mass spectroscopy experiments in shedding light on the structural stability of copper nanoclusters in solution. Furthermore, we will propose a plausible mechanism for copper transport across the blood-brain barrier, highlighting a potential pathway for restoring copper levels in central nervous system.

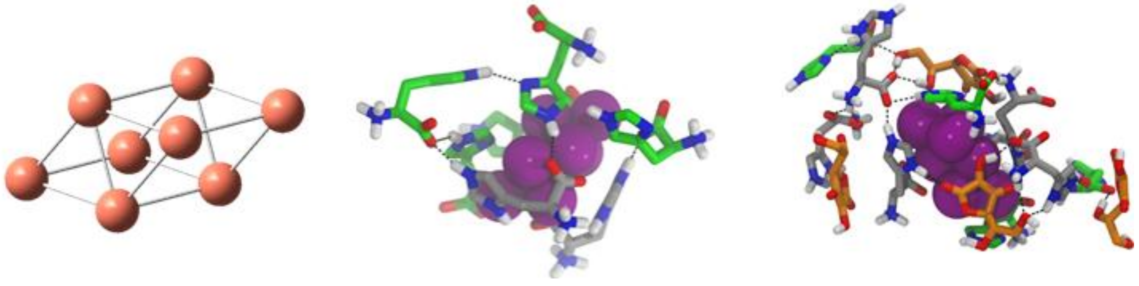

Fig. 1: Representation of the copper Nanocluster dans its surrounding stabilizing environment.

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Deciphering the Chemiexcitation Step of Luminol: A Computational Mechanistic Study

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Keywords: Luminol; Chemiexcitation; CASPT2

One of the main and frequently used chemiluminescent compounds is the molecule 5-amino-2,3-dihydrophthalazine-1,4-dione, better known as luminol. The importance of this molecule lies in its wide use in fields such as forensic chemistry, where it helps to detect traces of blood, and in biomedical research as a selective labeling technique. [1-3]

Over the years, numerous theoretical and experimental studies have been carried out to try to explain the chemiluminescence mechanism of luminol. However, to date, the molecular details of this mechanism remain unknown. In order to shed light on this fact, a theoretical study using advanced methods such as CASPT2 and CASSCF was carried out, by using the reduced bicyclic endoperoxide molecule (R-EP, Figure 1) as a representative intermediate in the reaction. [1,4]

Three different mechanisms were proposed (Figure 1) depending on the initial reaction: in mechanism 1, the reaction starts with the removal of the N_2, while in the case of mechanism 2, the peroxide bond is broken first, and finally, in mechanism 3, a concerted process is supposed.

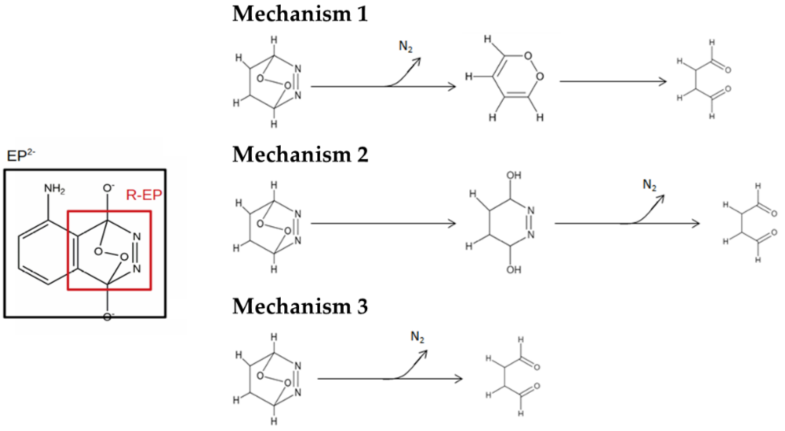

Figure 1. Proposed mechanisms for the reduced model of the bicyclic endoperoxide molecule of Luminol.

By optimizing the critical points for each of the mechanisms and by performing an exhaustive 2D scan of the potential energy surface, it was determined that mechanism 2 is impeded by the energetic barriers, while both mechanism 1 and 3 can contribute to the chemiluminescence process.

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Computational Inspection of Light-Sensitive Compounds Properties in Biological Systems

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Keywords: Photoresponsive; Membranes; Multiscale approaches.

One of the pivotal points of drug design concerns the synthesis of new molecules with minimal capability to induce harmful side effects in human organisms. Therefore, beyond exhibiting biological activity, the drug must possess high selectivity and very low toxicity as intrinsic properties.

In this context, light-dependent approaches represent a progressive advancement of conventional strategies used in medicine for several disorders, like cancer, neurological damage and infections [1][2]. In the range of UV-visible wavelengths, light is benign, clean and safe and finds applications in many scientific fields: from optical technologies that enable the modulation of different biochemical functions of cells in a spatiotemporal precise manner [3], to photodynamic therapy that induce space-specific cell death [4]. Different photoresponsive compounds have been recently proposed showing promising biological and therapeutic activities exploiting different mechanisms of action [5][6][7].

In this work, multiscale computational methods combining quantum and molecular mechanics, DFT/TDDFT and MD approaches respectively, have been used to give an insight into the chemical-physical properties of (i) a new generation of photoactuators able to induce neuronal photostimulation and (ii) newly synthesized photosensitizers exerting encouraging cytotoxic activity. Moreover, the modes of action have been explored by targeting biological membranes with a specific focus on the interaction between molecules and the lipid bilayer. All results will be presented along with the support and validation of the experimental data.

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Aknowlegments

PRIN D.D. 104/2022 PNRR M4.C2.1.1. – Project Code: 2022AN47CA – CUP: H53D23003820001, funded by the European Union – NextGenerationEU – Project Title "LA-G4-DACA" – Grant Assignment DD 862, 16/06/2023 is aknowledged.

DNA binding/unbinding kinetics of the dioxetane of melatonin

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Keywords: chemiexcitation; melatonin; DNA binding/unbinding; enhanced sampling molecular dynamics.

Melanin is a pigment that acts as a photoprotector due to its broad absorption spectrum. However, in melanocytes, it can also contribute to DNA damage; at least 50% of the cyclobutane pyrimidine dimers (CPDs) linked to UV-induced melanoma arise after irradiation via chemiexcitation, forming so-called "dark CPDs". This mechanism involves dioxetanes generated by oxidation of melatonin that, upon ring cleavage, release triplet excited state carbonyls capable of transferring energy to DNA, thereby generating genetic damage[1]. Molecules capable of forming dioxetanes under oxidative stress conditions, such as the DHICA monomer of melanin, are responsible for this damage in melanocytes. Melatonin may also possess this capacity, although it remains to be demonstrated [2].

Melatonin is an endogenous indolamine with strong antioxidant properties. Due to these benefits and its apparent lack of adverse effects under physiological conditions, it is considered a promising biomedical molecule [3]. However, it has been hypothesised that under inflammatory conditions it may increase DNA damage, similar to the DHICA monomer of melanin [2].

In the present work, computational simulation tools are used to investigate and characterise the kinetic profile of the interaction between melatonin-derived dioxetane and DNA, with the aim of determining its residence time and the molecular mechanisms of unbinding. This analysis allows us to evaluate whether the interaction time is sufficient for dioxetane chemical excitation and triplet energy transfer to nucleobases to occur, implying potential DNA damage. Classical molecular dynamics simulations were performed with GROMACS, complemented by enhanced sampling molecular dynamics using OPES flooding and PLUMED. These techniques allowed the identification of two modes of interaction of dioxetane with DNA: intercalation and double insertion. For each configuration, the residence time was analysed and the unbinding pathways were characterised, considering as main mechanisms the exit through the minor groove and major groove.

The funding for these actions/grants and contracts comes from the European Union's Recovery and Resilience Facility-Next Generation, in the framework of the General Invitation of the Spanish Government's public business entity Red.es to participate in talent attraction and retention programmes within Investment 4 of Component 19 of the Recovery, Transformation and Resilience Plan.

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Developmental Plasticity in Vertical Farms

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Keywords: Photobiology; Circadian; Controlled Environment Agriculture; Developmental Plasticity

Traditional crops exhibit considerable developmental plasticity in response to environmental cues such as light quality, photoperiod, and temperature fluctuations. In vertical farming systems, this plasticity can lead to variable growth rates, inconsistent flowering times, and unpredictable harvest schedules, undermining the economic viability of these operations. Recent advances in plant photobiology offer promising solutions through the development of crops with reduced environmental sensitivity.

Environmental control strategies complement genetic approaches by maintaining highly stable growing conditions that minimize triggers for developmental plasticity. Advanced LED lighting systems, precise climate control, and nutrient delivery optimization create environments where genetic modifications can be most effectively utilized. We will also describe genetic approaches to limiting developmental plasticity include manipulation of circadian clock components and photoreceptor signaling pathways to create plants with more uniform growth patterns and consistent flowering times across varied environmental conditions. These "timeless" crops maintain productivity while showing reduced responsiveness to light and temperature variations.

Future research directions include expanding these approaches to additional crop species, optimizing the balance between environmental responsiveness and productivity, and developing automated systems for monitoring and controlling developmental consistency in commercial vertical farming operations.

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The Role of Phytochrome Interacting Factors (PIFs) in Reducing Food Waste

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Keywords: Dark-induced senescence; UV light; PIF

Leaf senescence in the final stage of leaf development is a strategy to remobilise nutrients from older, photosynthetically limited tissues to developing parts of the plant. However, leaf senescence may also be induced by environmental stresses such as prolonged darkness [1] and is exacerbated by high ambient temperatures [2].

In commercial horticulture, this problem is exacerbated by the transport and storage of harvested produce in dark conditions, requiring producers and retailers to invest in costly and environmentally unfriendly refrigeration technologies to mitigate this problem. Leaf senescence not only limits produce distribution time and shelf life, but reduces the quality for end users. Methods to sustainably delay leaf senescence are a key objective for the fresh food industry, yet cost-effective and practical solutions remain limited.

In *Arabidopsis thaliana*, dark-induced leaf senescence is promoted by the light, temperature and the circadian clock-regulated transcription factors Phytochrome Interacting Factor 4 (PIF4) and PIF5 [3]. We have shown that low fluence UV-B light treatments can promote rapid degradation of PIF4 and PIF5 [4] proteins and suppress the expression of PIF4 and PIF5 genes in *Arabidopsis* [5]. This leads to a reduction in the expression of the key senescence regulator, ORE1 [3], and delays the onset of dark-induced senescence.

As UV-B is filtered by glass in commercial greenhouses and is not a component of horticultural lighting in vertical farms, UV-B is absent from the majority of protected growing environments used in commercial horticulture. We have found that in multiple species, a short duration, low fluence preharvest UV-B treatment can delay dark induced leaf senescence through a mechanism which is likely to involve PIFs. The delay in senescence can result in an extended shelf life for leafy produce.

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Translating academic photobiology to the context of a vertical farm

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Keywords: Vertical farming; CEA; Pulsing; Far-red

Light is critical for the normal growth of crops. In addition to serving as a source of energy to drive biomass production, several elements of light are also critical regulators of key agri and horticultural characteristics, such as the size of edible organs (e.g. leaves and tubers), the synthesis of volatile and secondary metabolites, as well as the production of flowers. Photobiologists have, consequently, long sought to understand how plants sense changes in their light environment and how, in turn, these changes influence growth. Thanks to advancements in Total Controlled Environment Agriculture (TCEA) technology over the last 15 years, it is now possible to utilise this understanding to guide the design of tailored lighting strategies that optimise crop development. Crucially, the precision of modern TCEA systems means that these strategies are not restricted to a focus on the needs of the plant, but also the desires of the grower and the practicalities of the system, leading to the prospect to test novel photobiological principles. Intelligent Growth Solutions (IGS) is a world leading developer and manufacturer of scalable vertical farms with customers located globally. A core benefit of IGS systems, designed in concert between plant scientists, software and hardware engineers, is the ability to control the intensity and spectral composition of light across 4 waveband channels. As a result, crop development can be precisely coordinated through targeted adjustments to the light environment. This, ultimately, not only enables consistent, year-round production of high quality produce for commercial growers, but the flexibility of IGS's technology also permits for cutting edge plant photobiology research to be conducted at the intersection of academia and industry.

In this talk, I will discuss the technology present within IGS systems, along with case studies of how IGS apply photobiological principles in the development of 'light recipes' focussed on improving the quality and production efficiency of vertically farmed crops. Examples of this include the integration of light wavebands, such as green and far-red light, that are often overlooked in many of the fixed spectrum LEDs commonly used in vertical farms, to improve whole canopy photosynthetic efficiency. I will also highlight how IGS are exploring novel avenues to adjust the volatile profile of crops grown in their farms, such as the use of high frequency pulsed lighting. Finally, I discuss challenges faced when translating promising findings from academic research to applied TCEA settings, such as managing trade offs between energy costs and potential yield gains, particularly given the impacts of high energy prices on businesses in the sector in the past 5 years. Nonetheless, the need to improve the commercial viability of vertical farms has resulted in the generation of solutions to real-world problems, as well as the exploration of new research avenues that push forward the photobiology field.

Controlling light-energy use in a minimal protein network to understand how plants regulate light harvesting

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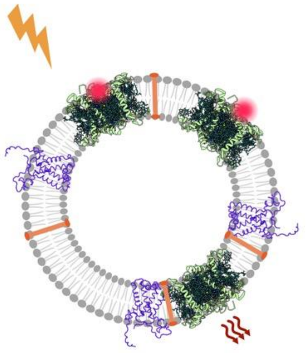
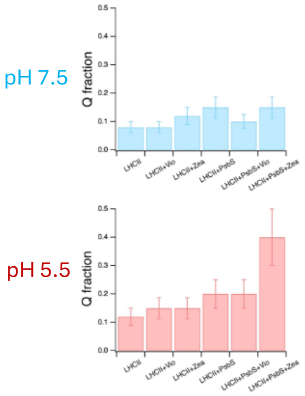
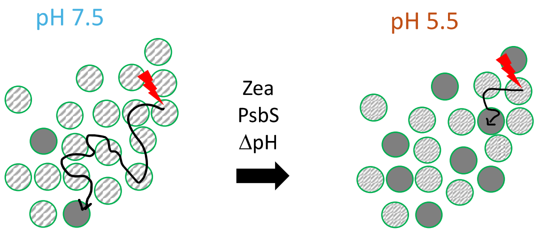
Keywords: Photosynthesis, Non-photochemcial Quenching, Xanthophylls, Thylakoid Membrane

A fine balance between photochemistry and photoprotection is crucial for plant fitness and determines the efficiency of light energy use for photosynthesis. Plant photoprotection in excess light is activated by acidification of the thylakoid lumen, activating the pH-sensing protein Photosystem II Subunit S (PsbS) and the conversion of violaxanthin (Vio) to zeaxanthin (Zea). The resulting processes act on the light-harvesting antenna, switching into a quenched state where photons are dissipated as heat [1]. To gain molecular insight into these photo regulatory processes, we succesfully reconstituted minimal protein networks and performed fluorescence lifetime analyses. (i) Regulatory light-harvesting activity by Light-Harvesting Complex II (LHCII) requires native thylakoid lipids and is modulated by pH, PsbS, and Zea -but not Vio. (ii) Point mutations in the luminal H2 and H3 loops of PsbS abolish its ability to enhance LHCII quenching, supporting the hypothesis that loop refolding is essential for PsbS function and suggesting its direct interaction with LHCII. (iii) PsbS and low pH increases the fraction of quenched LHCII (Q) from ~10% to 20%. Zea further increased Q to 40%, but only when PsbS was present, underlining the synergistic roles of Zea and PsbS in modulating excitation energy dissipation. These results align with whole-leaf fluorescence induction studies, validating the relevance of our model. We are currently exploring the effects of membrane lateral pressure and fluidity on light harvesting regulation by incorporating photo-responsive azolipids to modulate membrane tension and using fluorescent probes to monitor changes in membrane fluidity. Further, we aim to understand the molecular interactions by which Zea and PsbS synergically influence LHCII.







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Genetic Basis of Tillering Plasticity in Response to Plant Density in Sorghum

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Keywords: Tillering plasticity; Plant density; Red: far-red ratio; GWAS

Tillering responsiveness to plant density is critical for sorghum yield stability in water-limited environments, where controlled tillering enhances water use efficiency and drought tolerance [1]. This study aimed to dissect the genetic architecture of tillering responsiveness to neighbour spacing using planting density to induce different red: far-red (R:FR) light ratios [2,3]. A diverse sorghum panel (n = 895) was evaluated across two field environments over two growing seasons (2023–2024) at plant-to-plant spacings from 10 to 60 cm. A novel linear mixed model incorporating GPS-based neighbour distance measurements and tiller counts estimated genotype-specific response to neighbours. GWAS analysis was conducted on isolated plants (without neighbours) and estimates of plant responsiveness to neighbours. GWAS identified 52 tillering QTL from the isolated plants and 102 QTL associated with spacing responsiveness. Substantial overlap (27 QTL) between tillering in isolated and density-responsive QTL indicated shared genetic control mechanisms for both traits. This overlap is likely due to genes associated with self-shading. QTLs identified were compared with a set of 21 candidate genes, previously identified by homology to light signaling and phytochrome pathway genes from model species [4]. Significant enrichment was found with 40% of candidate genes co-locating with QTLs for responsiveness compared to 5% for isolated. This result indicates that the phenotypic model we used was able to separate the components of branching associated with light responsiveness and identified 67 novel QTL regions for this trait. This represents the first association study of spacing responsiveness in cereals, elucidating genetic mechanisms underlying density tolerance in sorghum. Tillering is a major controller of water-use in cereals [1]. Reducing a genotype's response to lack of neighbours could be used to develop varieties that perform well in water-limited environments [1,5].

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Bioenergetics of the cyanobacteria during mixotrophy

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Keywords: Synechocystis; Photosystem II; mixotrophy

ABSTRACT

The regulation of photosynthetic electron transport during photomixotrophic growth in cyanobacteria remains poorly understood. Here, we characterized four wild-type sub-strains of *Synechocystis* sp. PCC 6803 and found that prolonged photomixotrophy triggers a variable inhibition of Photosystem II (PSII) activity across strains. This inhibition is primarily attributed to impaired electron transfer from Q_A^- to Q_B, indicating a shift toward photoheterotrophic metabolism. Concomitantly, increased accumulation of the PSII-associated protein Psb28-2 suggests its role in modulating the Q_B-binding pocket, restricting forward electron flow while stabilizing PSII dimers. Strains retaining active photosynthesis showes higher glucose uptake but reduced growth. This study advances our understanding of the dynamic regulation of cyanobacterial photosynthesis and identifies potential targets for optimizing performance under different carbon regimes.

Photosynthesis regulation impacts carbon and nitrogen assimilation in the diazotrophic cyanobacterium *Anabaena* sp. PCC 7120

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Keywords: Cyanobacteria, Electron transport, Nitrogen fixation, Photosynthesis

Diazotrophic cyanobacteria fix both atmospheric carbon (C) and nitrogen (N) into biomass, but the two assimilation pathways are not compatible. Species like *Anabaena* sp. PCC 7120 physically separate C and N assimilation in different cell types. Even if separated, they are strongly intertwined, as N assimilation relies on the C skeletons and reducing power from photosynthesis, that in turn depends on N rich molecules as pigments and proteins.

Whereas the two pathways have been extensively studied individually, here we investigate their interaction by analysing photosynthetic properties upon exposure to changes in light, CO_2 and N availability, including the contribution of photosynthetic electron fluxes.

Growth depended on the availability of both light and CO_2, while the N_2 fixation activity mainly on the C supply. Upon diazotrophic conditions, the total photosynthetic electron transport activity increased, with a modified contribution of different electron pathways.

A mutant strain affected in the vehiculation of fixed N between cell types showed that the modulation of photosynthesis depended on the metabolic connection between assimilation pathways.

Overall, data showed that the regulation of photosynthetic electron fluxes is a major component of the synergic metabolic relationship between C and N assimilation pathways upon dynamic environmental conditions.

Aquatic photosynthesis in extreme temperatures

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Photosynthetic organisms in extreme environments have evolved diverse strategies to optimize light harvesting and energy transfer under environmental stress. We examine light-harvesting mechanisms in two ecologically distinct systems: the cold-adapted diatom *Chaetoceros simplex* from polar oceans and four species of Symbiodiniaceae, dinoflagellate symbionts central to coral reef health. Using steady-state and time-resolved fluorescence spectroscopy, we show that *C. simplex* maintains efficient energy transfer from fucoxanthin chlorophyll a/c proteins (FCPs) to photosystem I (PSI) and II (PSII), with no detached antennae. PSI lacks red-shifted chlorophyll fluorescence (>700 nm), yet exhibits a long decay time (~85 ps), indicative of a large core-antenna supercomplex. PSII shows lifetimes of ~500 ps (open state) and ~1220 ps (closed state), slightly slower than in temperate diatoms, suggesting larger antennae. Energy transfer is preferentially mediated by red-absorbing fucoxanthins (500–550 nm) over blue-absorbing ones.

Different Symbiodiniaceae species exhibit species-specific light-harvesting strategies under high light and temperature stress. While excitation energy transfer is ultrafast across all species, differences emerged in pigment content, PSI/PSII energy distribution, and fluorescence lifetimes. Under stress, species-specific changes in pigment composition and fluorescence kinetics were observed, highlighting dynamic regulation of light harvesting.

Together, these findings reveal that adaptations of photosynthetic machinery to extreme temperatures display finely tuned structural and functional adaptations in their photosynthetic apparatus. Such diversity underscores the ecological specialization and potential resilience of photosynthetic organisms to environmental extremes, from freezing polar waters to thermally stressed coral reefs.

Nanomaterials for enhanced photoprotection in photosynthetic microorganisms

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Keywords: carbon-based nanomaterials; high-light stress; photoinhibition; microalgae

Photosynthetic microorganisms, such as microalgae, are key contributors to global carbon fixation and hold great potential for applications in sustainable production of commodities and biotechnology. However, their efficiency is often limited by environmental stressors, particularly high light intensity, which induces photoinhibition, oxidative stress, and a consequent decline in overall performance. Advances in nanotechnology offer promising strategies to mitigate these effects and enhance organismal resilience [1]. This work investigates the use of carbon-based nanomaterials, specifically single-walled carbon nanotubes (CNTs) and nanodiamonds (NDs), as tools to improve photoprotection and maintain photosynthetic stability under stress, focusing on the green microalga *Chlamydomonas reinhardtii* [2,3]. The results indicate that the potential phytotoxic effects of nanomaterials can be minimized by optimizing their physicochemical properties and dispersion. Low concentrations of well-dispersed, purified, and small-sized CNTs or hydroxylated NDs did not negatively affect growth or pigment accumulation. Under photoinhibitory conditions, samples treated with CNTs showed reduced Photosystem II (PSII) inactivation, lower excitation pressure, higher rates of electron transport, and enhanced non-photochemical quenching compared to untreated controls [2]. In parallel, NDs exhibited effective ROS-scavenging properties and contributed to maintaining redox homeostasis under photooxidative conditions [3]. The precise mechanisms of interaction between carbon nanotubes and photosynthetic structures remain to be fully elucidated. Studies on the energy flow within these biohybrid systems have yielded conflicting results. To explore this further, we examined the electro-optical interactions of CNTs with isolated photosynthetic components of varying complexity. Our results suggest a possible leakage of photosynthetic electrons toward the nanotubes, most likely occurring at the PSII acceptor site, thereby facilitating non-radiative energy dissipation [4].

Overall, integrating nanomaterials with photosynthetic systems presents a promising strategy to enhance light use efficiency, safeguard against photoinhibition, and enhance the biotechnological potential of microalgae. The outcomes of this research highlight the potential of algal nanobiotechnology in developing advanced biohybrid systems aimed at the sustainable generation of bio-based products and environmental resilience.

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Photoprotection by non-photochemical quenching compensates for less cortical screening in pale versus melanic sympatric pairs of lichen species

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Keywords: Melanin; Photosynthesis; Screening pigments; Light stress adaptation; Usnic acid;

Lichens, symbiotic organisms of a fungus and a green alga and/or cyanobacterium, are vital components of extreme environments such as arctic, alpine, and dry ecosystems. To withstand severe abiotic stresses including intense light exposure, lichens employ various protective mechanisms. This study investigates the photoprotective mechanisms used by lichens in response to excessive light exposure, focusing on non-photochemical quenching (NPQ) and cortical screening.

We examined two pairs of sympatric lichen species. The first pair comprises the dominant pale usnic *Cladonia stellaris* and the patchier melanic *Cetraria islandica*, both found in open alpine heaths [1]. The second pair includes the melanic *Bryoria fuscescens*, typically located in the upper canopy of conifer trees, and the pale usnic *Alectoria sarmentosa*, which resides lower within these canopies in boreal forests [2]. Specimens from both pairs were collected simultaneously from sites with similar light exposure, enabling a direct comparison of their light-protective mechanisms.

Measurements of maximal photosystem II efficiency (Fv/Fm) following high light exposure demonstrated similar tolerance to excess light among species within each pair. The predominance of *C. stellaris* over *C. islandica* in alpine heaths exposed to high light is primarily attributed to its superior capacity for excess light dissipation via higher NPQ. Additionally, its pale surfaces contribute to the photoprotection by their high reflectance. Conversely, *C. islandica* employs melanic pigments for effective light screening. The niche separation of *B. fuscescens* and *A. sarmentosa* within the forest canopy reflects their distinct protective strategies. Positioned in the upper canopy characterized by stable and high light exposure, *Bryoria fuscescens*, benefits from efficient cortical screening due to decreased NPQ activity under dry, high light conditions in exposed upper canopies. Conversely, *A. sarmentosa*, located in the lower canopy, utilizes a high and dynamic NPQ to adapt to fluctuating light conditions and intermittent light flecks, aided by prolonged moisture retention.

In conclusion, pale lichen species containing usnic acid offset their reduced screening capabilities by employing a more robust and adaptable NPQ than to their melanic counterparts, facilitating their survival across varying light environments.

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Understanding the impact of photoprotection mechanisms on ROS regulation in the moss *Physcomitrium patens*

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Keywords: *Physcomitrium patens*; Reactive oxygen species (ROS); Photosystem I (PSI); Cyclic electron flow (CEF); Pseudo cyclic electron flow (PCEF).

To meet the increasing global demand for food, agricultural yields will need to rise by an estimated 60–110% by mid-century, while simultaneously reducing the environmental impact of agriculture. A new agricultural revolution is needed, one that harnesses cutting-edge genetic technologies with a particular focus on improving photosynthetic efficiency. Photosynthesis converts light into energy and represents the fundamental biochemical pathway that sustains plant life and other photosynthetic organisms. Regulatory mechanisms such as cyclic electron flow (CEF) and pseudo-cyclic electron flow (PCEF) play a key role in modulating photosynthetic electron transport and are essential for photoprotection under high and fluctuating light conditions, with a significant impact on growth [1,2]. We hypothesised that this is associated with the uncontrolled accumulation of reactive oxygen species (ROS), resulting in photodamage to photosystem I (PSI). This study aims to elucidate the role of CEF and PCEF in mitigating ROS accumulation by quantifying ROS production in *Physcomitrium patens* mutants lacking one or both photoprotective mechanisms and exposed to either constant high light or a fluctuating light regime. We also aim to investigate the impact of ROS accumulation on the redox regulation system of *P. patens*. By comparing these mutants to the WT, we expect to observe higher ROS levels in CEF or PCEF KO mutants followed by a significant impairment in Calvin–Benson cycle regulation.

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Interactive Effects of Ultraviolet Radiation and Elevated CO_2 on Estuarine Phytoplankton: Constant vs Variable Treatments

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Keywords: Photosynthesis, UV radiation, ocean acidification

EXTENDED ABSTRACT

Enhanced CO_2 in the aquatic environment affects phytoplankton physiology and metabolism. Increased atmospheric CO_2 triggers a decrease of cellular energy-consuming pathways, including those used to defend against acute stresses such as exposure to UV radiation. Phytoplankton cultures acclimated to high CO_2 under stable steady-state conditions are more susceptible to stress, but there is little study of this response in natural populations. Of particular interest are eutrophic systems such as estuaries that can experience strong diel cycling of aqueous CO_2 (pCO_2) with fluxes dominated by photosynthesis during the day and respiration at night. Seasonally, these systems can repeatably experience pCO_2 well above current atmospheric levels. We compared the responses to UV irradiation of estuarine phytoplankton assemblages under their natural conditions of varying pCO_2, versus constant low or high pCO_2 as typically used in laboratory studies. pCO_2 in microcosms was regulated by gas exchange with either ambient air (~400 ppm) or a 1000 ppm CO_2 mixture, or a variable CO_2 source from a specialized system (spherical falling film equilibrator [1]) that produces a gas mixture with CO_2 in equilibrium with the system of interest, the Rhode River, an estuarine inlet of the Chesapeake Bay, USA [2]. Over a ten-day experimental period in July, 2022, the average pCO_2 levels were 439 ppm in the ambient treatment, 1104 ppm in the high treatment and 1106 ppm in the equilibrium treatment. However, in the equilibrium treatment there was diel variation between a maximum of 2300 ppm in the morning to minimum of 314 ppm at day's end, matching levels occurring in the river [2]. At the beginning of the experiment, laboratory assays showed a high sensitivity of photosynthesis to inhibition by UV radiation, similar to previous studies with Rhode River natural assemblages. Over the course of the experiment, sensitivity to inhibition remained high in the equilibrium assemblage but decreased by ~ 40% in *both* the ambient and high treatment. The decrease in sensitivity coincided with a slowing growth rate in the constant treatments contrasting with rapid growth in the equilibrium treatment. The results are consistent with the general concept that phytoplankton prioritize growth under favorable conditions but under unfavorable conditions augment defense systems that increase resistance to UV exposure, among other effects. In addition, the results suggest that experiments using constant CO_2 treatments may not produce results that are representative of the responses of phytoplankton assemblages in systems with diel varying pCO_2.

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PUVA – Still a Valuable Option for Skin Disease?

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Keywords: PUVA, Biologics, Efficacy, Adverse Effects.

Psoralen – UVA (PUVA) is a well-established, but sometimes forgotten, therapeutic option for a wide range of skin diseases. It has undoubted efficacy in many conditions and whilst narrow band UVB phototherapy would usually be the first phototherapeutic option for most skin diseases for whom topical therapies do not suffice, the availability of PUVA is invaluable for patients who either fail to respond or rapidly relapse after UVB phototherapy[1]. Indeed, it would be a first line phototherapeutic option for patients with hand and foot dermatoses, pityriasis rubra pilaris, plaque mycosis fungoides, hypertrophic lichen planus or nodular prurigo. For psoriasis, studies have indicated >75% in psoriasis severity in approximately 80% of patients, comparable to overall outcomes with biologic treatments. If psoriatic arthritis is a factor, PUVA would not be suitable as a systemic approach is required.

Historically, the skin cancer risk of PUVA has instilled undue concerns for some, but risk is mainly for those having had >150 PUVA treatment and keeping this in context, a course of PUVA may only involve 12-16 treatments, and with monitoring and avoidance of maintenance treatment, most patients will never reach such high cumulative treatment numbers. Furthermore, this must be balanced against adverse effects with other therapies, including the biologics. PUVA may be preferred for patients with localised disease or those with an acute flare-up but may not need long-term treatment, such as with biologics. In an established phototherapy unit, the practical aspects of PUVA delivery should not be limiting. PUVA is typically more cost-effective than biologics, although biosimilars, insurance cover, reimbursement and practicalities of service set ups will play a part.

In conclusion, PUVA remains an invaluable option for the management of a variety of skin diseases. Individual factors will come into play when deciding on the most appropriate option for patients, such as their diagnosis, disease severity, comorbidities and patient and service preference. Retention of PUVA services is an asset for dermatology, enhancing the availability of personalised patient-centred care and effective therapeutic options.

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Phototherapy of atopic dermatitis

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Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disorder characterized by intense pruritus and a significant impact on quality of life. While topical and systemic treatments remain the cornerstone of management, phototherapy has emerged as a valuable, evidence-based therapeutic option, particularly for moderate-to-severe and refractory cases. This presentation will provide an updated overview of the mechanisms, efficacy, and safety of various phototherapeutic modalities in AD, including narrowband UVB and UVA1. Special emphasis will be placed on patient selection, treatment protocols, long-term outcomes, and integration into personalized therapeutic strategies. Recent insights into the immunomodulatory effects of phototherapy and its role in the era of biologics will also be discussed. Phototherapy continues to be a clinically relevant, cost-effective, and well-tolerated approach in the multidisciplinary management of atopic dermatitis.

Phototherapy for psoriasis at the age of biologics (originators and biosimilars)

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Despite the advent of highly effective biological agents, phototherapy (PT) remains a well-established treatment for psoriasis. PT, which nowadays primarily involves narrowband UVB (much more than PUVA), is effective in treating all subtypes of psoriasis, including guttate, erythrodermic and pustular manifestations of the disease, such as palmoplantar psoriasis. Although PT offers intermittent treatment schedules, it still requires a significant time commitment, involving multiple clinic visits (i.e. one treatment cycle consists of two to three treatments per week over six to ten weeks). PT is also suitable for pediatric patients, pregnant or lactating women, or individuals with malignancies. PT is compatible with all vaccinations and can be administered to patients with infections such as latent tuberculosis and HIV. Interestingly, PT carries no risk of secondary loss of response, and remission typically lasts 6–12 months. PT is a multi-target treatment that leads to the direct apoptosis of keratinocytes and immune cells, as well as immune suppression through the induction of regulatory T cells (Tregs), among other mechanisms. In contrast, biologics are mostly monotargeting, are primarily indicated for moderate-to-severe plaque psoriasis and offer rapid disease control and minimal time expenditure. They also provide additional benefits for nail and scalp involvement, as well as possibly some psoriasis-associated comorbidities. However, limitations include contraindications in pregnancy, infectious diseases and inflammatory bowel disease (for IL-17 inhibitors), higher treatment costs and rare but potentially life-threatening adverse events. Although biologics currently dominate due to their convenience and efficacy, phototherapy remains a safe, cost-effective and valuable option for certain patient groups, particularly where biologics are contraindicated or inaccessible.

Phototherapy of vitiligo

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NB-UVB phototherapy has been the mainstay of the treatment of vitiligo for almost 20 years.

Several studies have also demonstrated that the efficacy can be improved by the concomitant application of khellin, phenilalanyne, corticosteroids and topical calcineurin inhibitors like tacrolimus and pimecrolimus.

Recently, both topical and oral JAK inhibitors like including ruxolitinib, baricitinib, and tofacitinib have demostrated a good efficacy and more than 10 other molecules are investigated in phase 1, 2 and 3 trials.

However, other studies have demonstrated that the efficacy of JAK inhibitors can be significantly improved by the combination with NB-UVB phototherapy, particularly for lesions of the face.

In addition, the combination seems much more cost-effective than the drug treatment alone.

We report and discuss our results.

However, we have not a trial so far comparing a JAK inhibitor alone with phototherapy alone so far.

Omalizumab therapy for solar urticaria is associated with behavioural and quality-of-life benefits

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Keywords: solar urticaria, photodermatosis, omalizumab, quality of life, sun exposure behaviour

Background: Solar urticaria is a debilitating photodermatosis triggered by exposure to ultraviolet (UV) radiation and/or visible light. Due to the severity of photosensitivity, individuals often adopt extreme behavioural adaptations to avoid light exposure, significantly impairing quality of life. While the anti-IgE monoclonal antibody omalizumab has demonstrated efficacy in improving several clinical outcomes, its impact on behavioural measures, particularly those related to daylight exposure, remains poorly characterized. This study aimed to evaluate changes in daylight-related behaviour and quality of life before and after omalizumab therapy in patients with solar urticaria.

Methods: Daylight exposure diaries were completed daily by n=5 patients with solar urticaria and n=7 healthy participants for one month during different seasons in the United Kingdom. The diaries captured detailed information including time spent outdoors, weather conditions, and clothing worn (indicating skin surface area exposed). Patients also completed Dermatology Life Quality Index (DLQI) questionnaires where higher scores indicate a greater impact on quality of life.

Results: Prior to omalizumab treatment, patients spent significantly less time outdoors during sunny conditions compared to non-sunny conditions in spring (mean 10 vs. 29 minutes/day, $p<0.05$), and spent less time outdoors than healthy volunteers (44 minutes in sun/day). However, following omalizumab therapy, patients demonstrated a substantial increase in time spent outdoors under sunny conditions, with a mean springtime exposure of 45 minutes/day, comparable to that of healthy volunteers. Further increases were observed in summer (mean 85 minutes/day) and autumn (39 minutes/day). These behavioural changes were accompanied by increased skin surface area exposure (pre vs post omalizumab in spring: 7% vs 11%), and a reduction in the occurrence of symptoms when outdoors, with symptoms occurring 75% days in spring pre-omalizumab vs 26% days in summer on omalizumab. Quality of life improvements were demonstrated by significant reduction in DLQI scores (mean score 22 pre-treatment vs 5.8 post-treatment in summer, $p<0.001$).

Conclusion: Omalizumab therapy in patients with solar urticaria is associated with measurable, meaningful behavioural alteration, including increased daylight exposure and reduced protective clothing use, alongside reduced symptoms and improvements in quality of life. These findings support the value of including behavioural measures as important outcome domains in assessing therapeutic response, and further highlight the benefit of omalizumab therapy.

Acknowledgement: This study was jointly funded by the National Institute for Health and Care Research (NIHR) Manchester Biomedical Research Centre (BRC) (NIHR203308) and the British Skin Foundation. The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care.

Identifying new markers that predict the effectiveness of photoprotective agents in reducing skin carcinogenesis

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Keywords: photoprotection, photocarcinogenesis, vitamin D, biomarkers

Ultraviolet radiation (UV)-induced skin carcinogenesis is primarily driven by UV-induced DNA damage, some of which is poorly repaired, leading to mutations, and UV-induced immune suppression, which hampers the immune system's ability to recognize and eliminate developing skin tumours. Our research has demonstrated that the vitamin D hormone, 1,25-dihydroxyvitamin D (1,25D), applied immediately after UV exposure, reduces DNA damage, immune suppression, and skin tumour development. Vitamin D-like compounds such as 1,25-dihydroxylumisterol and tetrahydrocurcumin show similar protective effects. Interestingly, while several vitamin D-like compounds and other potential photoprotective agents we have tested reduce UV-induced DNA damage and immune suppression in acute studies, they have not shown a reduction in photocarcinogenesis in a well-established 40-week murine model. This highlights the need for reliable, easily measurable markers, beyond DNA damage and immune suppression, that can identify agents capable of reducing skin tumours in mice exposed to chronic UV.

We further investigated the photoprotective effects of vitamin D compounds in primary human skin cells, Skh:hr1 mice, and *ex vivo* human skin, with the aim of developing a framework to identify which markers, or combinations of markers, best predict an agent's ability to reduce UV-induced skin tumours. Phosphatase and tensin homolog (PTEN) and N-myc downstream regulated gene-1 (NDRG1) are protein markers 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 melanocytes and in Skh:hr1 mouse skin, but significantly increased with 1,25D treatment. Furthermore, we demonstrated that phosphorylation of cyclic AMP response element binding protein (CREB), which has been linked to carcinogenic potential, is increased in skin cells after UV and can be modulated by vitamin D compounds. Interleukin-6 (IL-6) is an inflammation marker that is increased following UV exposure, while IL-10 also increases following UV and contributes to immune suppression. We have demonstrated reductions in both of these cytokines with 1,25D.

Testing the efficacy of photoprotective agents against UV-induced skin tumour development is crucial for developing bioactive sun protection agents as an alternative to the simple UV filtration offered by sunscreens. The process, however, is lengthy (taking up to 40 weeks), labour-intensive, and costly. If there were early markers to predict efficacy and identify agents unlikely to reduce tumours, it would significantly expedite the identification of promising candidates for this extensive testing.