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# A new approach to targeted PDT using upconversion nanoparticles and a highly specific DARPin

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**Keywords:** upconversion nanoparticles; DARPin; targeted therapy

The use of nanoparticles for the targeted delivery of light-sensitive agents or therapeutic drugs to cancer cells is a promising approach for highly selective photodynamic therapy. For large tumours, this approach can be improved by using nanoparticles that are able to transfer energy through upconversion. The lanthanide ions in the core of the nanoparticles absorb energy from the near infrared (NIR) and then emit it in the visible spectrum. This emitted energy is then absorbed by a photosensitiser on the surface of the nanoparticle, enabling a therapeutic effect [1].

In this study, we used customised core-shell upconversion nanoparticles carrying verteporfin, the photosensitiser commonly used in photodynamic therapy. The particles were doped with $Tm^{3+}$ and $Yb^{3+}$ to maximize the activation of verteporfin by NIR. The surface of the particles was chemically modified with polymers for efficient photosensitizer binding and functionalization with the bivalent Designed Ankyrin Repeat Protein (DARPin) to improve the specificity of targeting cancer cells. DARPin was specifically designed to bind to HER2 receptors, which are overexpressed on the surface of breast and colon cancer cells [2]. The conjugation of DARPin with the nanoparticles was analysed using spectroscopic techniques. The specificity and recognition of the functional nanoparticles were confirmed by flow cytometry with verteporfin fluorescence in SKBR3 breast cancer cells. The distribution of the new verteporfin carrier system in SKBR3 cells was observed by confocal fluorescence microscopy. This showed that the particles were rapidly recognised by the cells' HER2 receptors, leading to increased uptake of verteporfin as early as 1 hour after administration, which was not the case with non-functionalised nanoparticles containing verteporfin.

In summary, this study advances the development of targeted photodynamic therapy through the successful conjugation of a specific DARPin with upconversion nanoparticles loaded with a photosensitiser. The results confirm the efficacy of this approach and form the basis for future experiments aimed at improving the stability of the construct and optimising its therapeutic potential in photodynamic treatments.

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The authors express gratitude to Professor Andreas Plückthun from UZH in Zurich, Switzerland, for supplying the plasmid of bivalent DARPin and acknowledge funding from the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project BCOrgFluorIDA No. 09I03-03-V04-00007.

# Exploring Fungal Photosensitizers for Targeted Cancer Therapy

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**Keywords:** Photodynamic Therapy; Natural pigments; *Talaromyces islandicus*; OSMAC

A major risk of conventional chemotherapeutics is their lack of selectivity for malignant cells, often leading to severe side effects on healthy cells. In Photodynamic Therapy this problem is avoided by using non-toxic drugs, whose phototoxic effect can locally be triggered by light; so called photosensitizers.

Anthraquinones, such as those found in the genera *Hypericum* [1], *Penicillium* [2], and other sources, are often colorful pigments whereas some of them are known photosensitizers. *Penicillium* and *Talaromyces* species are of particular interest due to their ease of cultivation, making them ideal for commercial pigment production [3].

The presented project aims to identify and characterize fungal photosensitizers that selectively kill cancer cells and can be cultivated on a large scale. The metabolites will be tested for their photocytotoxic effects on cancer cells, specifically from the nasopharynx and bladder, as these tissues allow for minimally invasive illumination. Key cell lines to be tested include T24 (*urinary bladder carcinoma)* Cal27 (squamous cell carcinoma) TE-1 (esophageal carcinoma), and RPMI 2650 (nasal septum carcinoma).

To identify promising metabolites, the OSMAC (One Strain Many Compounds) approach was used, which suggests that a single microbial strain can produce diverse metabolites under different cultivation conditions [4]. To stimulate the production of potentially photoactive anthraquinones, about 20 species of *Penicillium* and *Talaromyces* have been selected and cultivated under varying conditions.

Here we will present our preliminary results indicating that the extract of *Talaromyces islandicus* CBS 117284, already has a significant cytotoxic effect on cells at concentrations as low as c = 0.6 µg/mL, with enhanced toxicity following 15-minute blue light irradiation ($\lambda$ = 478 nm, H = 9.3 J/cm²). Putative active metabolites were annotated employing HPLC-DAD-MS analysis as well as NMR Spectroscopy and will be shown.

This research was generously supported by the Austrian Science Fund (P37163) and the University of Innsbruck.

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**Photodynamic Therapy and Diagnosis**

# Comparative Biocompatibility of Far-UVC and UVC Irradiation with Red Blood Cells

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**Keywords:** Far UVC light; UVC light; Red blood cells; Haemolysis

**Background:** Far-UVC light (200-230 nm) holds potential for use in tissue-contact decontamination applications, such as wound treatment, owing to recent research demonstrating its antimicrobial efficacy and enhanced safety in comparison to longer-wavelength UV light due to its limited penetration in human tissue [1]. The compatibility of far-UVC light with human skin and eye tissues is documented; however, its compatibility with whole blood and its components, which may be exposed during wound treatment, is broadly unknown. **Aims:** This study investigates and compares the biocompatibility of far-UVC light (~222nm), compared to longer wavelength UVC light (~254nm), with red blood cells in whole blood suspensions using two haemolysis quantification methods. **Methods:** Whole blood samples (25% PCV; 1.4 mL) were exposed to clinically-relevant antimicrobial doses of 222-nm or 254-nm light (≤18 J/cm$^2$), centrifuged (2200 × g for 10 min), and plasma-free haemoglobin – as an indicator of haemolysis– was measured using the Harboe spectrophotometric method [2] with Allen Correction [3] and Drabkin's spectrophotometric method [4]. **Results:** Both far-UVC and UVC light exposures exhibited dose-dependent haemolytic effects, with significantly greater ($P \leq 0.05$) levels of haemolysis indicated upon exposure to the former: statistically ($P \leq 0.05$) greater increases were demonstrated in comparison to non-exposed controls after 1.5 J/cm$^2$ exposure to far-UVC, versus 18 J/cm$^2$ exposure to UVC. Results also demonstrated that, whilst trends were consistent, Drabkin's spectrophotometric method detected significantly higher absolute haemoglobin values ($P \leq 0.05$) than that of the Harboe spectrophotometric method in all instances, suggesting the former may be more suitable for evaluation of oxidative modifications following light exposure. **Conclusions**: This study has successfully characterised the comparative biocompatibility of far-UVC and UVC with red blood cells in whole blood suspensions. Further, whilst both haemolysis quantification methods are suitable for plasma-free haemoglobin measurement, Drabkin's spectrophotometric method may yield more accurate values than the Harboe spectrophotometric method in the context of light-induced damage, given it captures all haemoglobin released by haemolysis, as opposed to measuring specific haemoglobin derivatives, which may undergo photodynamic modifications as a result of light-exposure (as evidenced by slight shifts in the spectral profile following light treatment). Whilst far-UVC remains a promising tool for tissue-contact decontamination applications, further work is necessary to optimise light exposure conditions such to preserve blood product integrity whilst ensuring antimicrobial efficacy.

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# Verification of the safety of antimicrobial photodynamic and sonodynamic therapy in a model system of reconstructed human skin

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**Keywords:** photodynamic therapy, sonodynamic therapy, safety

**Introduction**: Resistance to antibiotics and biocides is an escalating threat to the treatment and prevention of infectious skin diseases. Photodynamic therapy (PDT)and sonodynamic therapy offers alternative methods for treating local microbial infections based on the photodynamic phenomenon. This research aims to develop and apply new therapeutic strategies using PDT to reduce the necessary use of antibiotics and disinfectants. The safety and efficacy of combining different generation sensitizers with visible light of corresponding wavelengths were monitored on selected microbial strains using biophysical, microbiological, and in vitro methods in model systems simulating human tissues.

**Methods**: An in vitro model system of reconstructed human skin was used to verify the safety and efficacy of PDT and SDT. This method utilizes reconstructed models of human epidermis, optimized and adapted based on the validated in vitro phototoxicity method for chemicals (OECD TG 498), to identify the phototoxic potential of tested substances triggered by light exposure. Additionally, a skin irritation test was conducted using 3D models (OECD TG 439). The antibacterial effect of PDT in vitro was studied using the bacterial strains Staphylococcus aureus (S. aureus). We evaluated the sensitizer methylene blue (MB) activated by appropriate light wavelengths and high-frequency ultrasound on S. aureus using biophysical and microbiological in vitro methods. The antibacterial action of MB was assessed using the microdilution method and by subculturing bacteria on agar plates to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

**Results**: Our results show the MIC and MBC of MB for S. aureus when applying PDT at a light dose of 30 J/cm², with concentration values 0.78 μM. When PDT was applied at a double light dose (2x 30 J/cm²), these concentrations were further reduced to 0.195 μM, and with the combined application of PDT and SDT, to 0.14 μM. In conclusion, MB demonstrate significant antibacterial effects when used with PDT or in combination with PDT and SDT. The results from verifying the safety of PDT and SDT on the in vitro reconstructed human skin model indicated that selected sensitizers, at chosen concentrations and in combination with radiation at wavelengths corresponding to the sensitizer's absorption maximum in the visible spectrum, at an energy density of 2x 50 J/cm², did not reduce viability below 90%. In terms of safety verification, no skin irritation or phototoxicity was observed under the selected irradiation parameters combined with the selected sensitizers, which had already shown antibacterial effects on bacterial strains. Based on these results, all tested factors can be considered safe.

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# Exploring the Potential of Gallium Porphyrin for Intracellular *S. aureus* Targeting in an Atopic Dermatitis Cell Model

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**Keywords:** aPDI, GaCHP2-3, Atopic dermatitis, *Staphylococcus aureus*.

The intracellular persistence of bacteria constitutes a survival strategy within host cells and is associated with the development of chronic and recurrent infections. *Staphylococcus aureus* is a critical pathogens characterized by the production of multiple virulence factors and resistance to the numerous antibiotics [1]. Treatment of staphylococcal infections has become increasingly challenging, partly due to *S. aureus* ability to invade the nonprofessional phagocytes such as keratinocytes or fibroblasts. Atopic dermatitis (AD) is undoubtedly a condition where the staphylococcal persistence is linked to exacerbation of the disease. Moreover, the atopic phenotype such as deficiency of antimicrobial peptides or decreased levels of filaggrin facilitate the invasiveness of *S. aureus* which makes them also the major bacterial pathogen within AD patients [2]. The aim of this study was to evaluate the potential of the novel gallium porphyrin derivative GaCHP-2-3 in antimicrobial photodynamic inactivation (aPDI) of intracellular *S. aureus* in AD cell model. To do this, we explored the GaCHP-2-3's antimicrobial efficacy, cellular accumulation, and safety profile *in vitro*. The overarching goal was to assess the therapeutic potential of this approach in the management of recurrent skin infections, such as those encountered in the AD.
Antimicrobial susceptibility and photodynamic inactivation of *S. aureus* were assessed by incubating bacterial suspensions with serial dilutions of GaCHP-2-3, followed by irradiation ($\lambda_{max}$ = 409 nm and 522 nm) and CFU counting. The minimum bactericidal concentration (MBC) was defined as the lowest concentration yielding ≥99.9% bacterial reduction. The GaCHP-2-3 accumulation was evaluated in *S. aureus* and in two human keratinocyte cell lines, (HaCaT), namely shC/shFLG representing the control group and filaggrin knockdown, respectively. To do this varying concentrations and incubation times were utilized. The overall assessment was followed by fluorescence microscopy imaging, cytometry, and CFU analysis. Cytotoxicity and phototoxicity in shC/shFLG cells were monitored in real-time using the xCELLigence system, measuring cell viability *via* impedance-based Cell Index (CI) over 90 hours.
Our study shows that GaCHP-2-3 exhibits strong bactericidal activity and accumulates in *S. aureus*, thus enabling effective *in vitro* photoinactivation [3]. Additionally, GaCHP-2-3 was shown to accumulate in both shC and shFLG cells, while exhibiting no cytotoxicity and minimal phototoxicity at therapeutic doses. These findings highlight GaCHP-2-3 as a promising porphyrin derivative for potential use the therapy of skin infections in AD patients.

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# 2D and 3D heterotypic tumor models: What will be the difference in efficacy of photodynamic therapy with phthalocyanine derivatives?

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**Keywords:** photodynamic therapy, 3D model, spheroid, phthalocyanine

This project utilizes 3D heterotypic cellular tumor models suitable for the determination of the photodynamic activity of novel phthalocyanine photosensitizers. 2D cellular models, which are affordable and suitable for high-throughput screening, and still the gold standard in experimental practice. However, they cannot mimic the complex interactions between cancer cells and the surrounding microenvironment in the human body. The use of 3D models that partially mimic the physiological microenvironment may be a possible solution. 3D heterotypic tumor models can explain more complex mechanisms and show promise as a tool for drug development. Nowadays, they are used as systems to investigate different types of tumors due to the ability to reproduce the basic characteristics of solid tumors, especially metastases. However, common limitation of these models is usually the absence of vascularization [1]. Our cellular models consist of tumor cells (tGFP-HeLa, tGFP-CT-26), endothelial cells (RFP-HUVECs) and fibroblasts (MRC-5). Multicellular tumor spheroid (MCTS) development (on ULA plates) was analyzed by microscopic methods. Based on the growth curves, spheroids derived from CT-26 and HeLa cells in the day 3 post seeding (250 μm size) were chosen to study photodynamic activity. The amphiphilic cationic PS showed the highest phototoxicity against all cell lines. MCTSs showed higher resistance to treatment expressed by higher concentrations required to reach $IC_{50}$. The toxicity of cytostatic drugs (cisplatin, paclitaxel, irinotecan, 5-FU; 24 and 48 h incubations) was also tested in the same setup with MCTSs showing increased resistance to the treatment compared to monolayers. Our results are therefore in agreement with those reported in the published literature [2,3]. Viability was measured by resazurin assay. Preliminary experiments measuring the growth curves of heterotypic spheroids showed that spheroids composed of HeLa cells and HUVECs (1:1) grew faster, and by day 10 heterotypic MCTSs reached a diameter of 1500 μm, while HeLa homotypic MCTSs reached only 1000 μm. MCTSs derived from the CT-26 cell line seeded in extracellular matrix protein hydrogel displayed invasive potential. We observed a reduction of invasiveness after irradiation. However, there is still a lot of work to be done on this project, especially in the development of 3D heterotypic models consisting of more cell types.

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Photodynamic Therapy and Diagnosis

# Optimising narrow waveband phototesting for photosensitivity screening: A retrospective wavelength sensitivity analysis

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**Keywords:** Phototesting, Photosensitivity, Photodermatoses, Diagnostic Screening

**Background:**
Narrowband phototesting remains the diagnostic benchmark for evaluating cutaneous photosensitivity, encompassing the ultraviolet B (UVB), ultraviolet A (UVA), and visible light spectrum (300–600 nm) in the UK[1]. While diagnostically robust, its implementation is confined mainly to tertiary centres due to logistical complexity. Optimising waveband selection may enable the development of a reduced phototesting protocol suitable for wider clinical application, including secondary care screening.

**Objectives:**
To assess the diagnostic sensitivity of individual and combined wavebands in detecting photosensitivity and to determine the efficacy of simplified waveband combinations for broader clinical screening.

**Methods:**
A retrospective analysis was conducted on 216 consecutive patients with confirmed abnormal phototest responses assessed between 2021 and 2024 at a UK national photobiology centre. Patients underwent testing at five discrete wavebands: 305 nm (UVB), 335 nm (UVB/UVA), 365 nm (UVA), 400 nm, and 430 nm (visible light). Diagnostic coverage was evaluated for individual wavelengths and dual and triple combinations. Clinical diagnoses and Fitzpatrick skin phototypes were also analysed.

**Results:**
Sensitivity to individual wavebands was highest at 335 nm (74%), followed by 365 nm (71%), 305 nm (63%), 400 nm (39%), and 430 nm (16%). Diagnostic yield improved with dual combinations: 305 nm + 365 nm identified 89% of cases, while 335 nm + 365 nm and 305 nm + 335 nm detected 87% and 84%, respectively. Combinations involving visible light were slightly less effective, with 305 nm + 400 nm, 335 nm + 400 nm, and 365 nm + 400 nm detecting 78%, 81%, and 76% of cases, respectively. The highest overall sensitivity was achieved using the triple-waveband combination of 305 nm, 365 nm, and 400 nm, covering 96% of photosensitive individuals.

**Conclusion**
Combining multiple phototesting wavebands significantly improves detection rates of photosensitive individuals. Almost 90% of photosensitivity would be detected if screening with UVB and UVA wavelengths (305 nm+365 nm), which could be improved to 96% by combining with 400 nm. Limited wavelength phototesting (305 nm+365 nm+400 nm) has the potential to be a useful screening approach for photosensitivity. Careful wavelength selection could help identify at-risk individuals in secondary care settings before referral to tertiary centres.

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# Charged (Aza)phthalocyanine Photosensitizers for Antimicrobial Photodynamic Therapy focused on the ESKAPE group

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**Keywords:** photodynamic therapy; photosensitizer; phthalocyanine; ESKAPE

Antimicrobial resistance (AMR) is a growing global health concern, driven largely by the overuse and misuse of antibiotics. As bacteria evolve mechanisms to evade antibiotic effects, infections become harder to treat, leading to prolonged illness and increased mortality. One major contributor to AMR is the formation of biofilms—structured communities of microorganisms encased in a self-produced matrix that adheres to surfaces. Within biofilms, bacteria exhibit enhanced resistance to antibiotics and immune responses, making infections especially persistent and difficult to eradicate. This highlights the urgent need for alternative strategies to combat resistant pathogens [1, 2].
Antimicrobial photodynamic therapy (aPDT) is an emerging, non-invasive treatment strategy that offers a promising alternative to traditional antibiotics, especially in the fight against resistant microorganisms. It involves the use of a photosensitizer (PS)—a light-activated compound—that, upon exposure to a specific wavelength of light, produces reactive oxygen species (ROS). These ROS cause oxidative damage to microbial cells, leading to their destruction. Unlike antibiotics, aPDT has a broad-spectrum effect and does not rely on specific molecular targets, reducing the likelihood of resistance development. aPDT is particularly effective against biofilms, which are notoriously resistant to conventional treatments, making it a valuable tool in managing chronic and hard-to-treat infections [3, 4].
In this work, we studied novel (aza)phthalocyanine PSs bearing either anionic or cationic charges. Methylene Blue was used as a reference PS in all *in vitro* experimental setups. MRSA (methicillin-resistant *Staphylococcus aureus*), PA (*Pseudomonas aeruginosa*), CA (*Candida albicans*) were selected as model microorganisms for initial assessments. Antimicrobial photodynamic activity was determined by recovery method (Bioscreen C), confirmed by resazurin assay and spread plate method. Amphiphilic cationic PS was the most active derivative capable of complete eradication of MRSA after irradiation in very low doses (≤ 100 nM; but also, PA and CA using higher concentrations). aPDT activity was also assessed using clinical isolates found in chronic wounds (members of the ESKAPE group). Low toxicity against normal human cells (fibroblasts, keratinocytes and endothelial cells), negative hemolysis test (human red blood cells) and transdermal permeation (human skin) were assessed as well to give an insight into the safety of these novel compounds. Further research is underway, particularly to determine the antibiofilm activity and safety of all derivatives.

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# Plasmonic Mesoporous Silica Nanoarchitectures for the Chemo-Photothermal Targeted Treatment of Colorectal Cancer

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**Keywords:** plasmonic nanoparticles, mesoporous silica nanoparticles, photothermal therapy

## EXTENDED ABSTRACT

Plasmonic $Cu_{2-x}S$ nanocrystals (NCs), with tunable localized surface plasmon resonance (LSPR) in the NIR region, are promising agents in cancer photothermal therapy (PTT). Combining hyperthermia with chemotherapy enhances therapeutic efficacy. Functionalizing plasmonic nanoparticles with mesoporous structures enables synergistic drug delivery and PTT [1]. Mesoporous silica nanoparticles (MSN) are attractive due to their high surface area, pore volume, biocompatibility, loading capacity and ease of functionalization. Here, multicomponent core@shell nanostructures (MSN@MSN CR), featuring a mesoporous silica core embedding $Cu_{2-x}S$ nanocrystals and a dahlia-like mesoporous silica shell loaded with paclitaxel were designed for colorectal cancer therapy. Plasmonic $Cu_{2-x}S$@MSN@MSN CR, synthesized by a biphasic soft-templating approach, were surface modified with pH-sensitive polyacrylic acid and anti-PDGFRβ antibody for tumor targeting. A comprehensive morphological and photothermal analysis, along with drug loading and pH-dependent release assays, was conducted. *In vitro* studies on normal and cancer colon, gastric and pancreatic cell lines were performed, demonstrating a strong potential of $Cu_{2-x}S$@MSN@MSN CR for targeted, synergistic chemo/phototherapy.

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# Development of a Transient Absorption Imaging System (TAIS)

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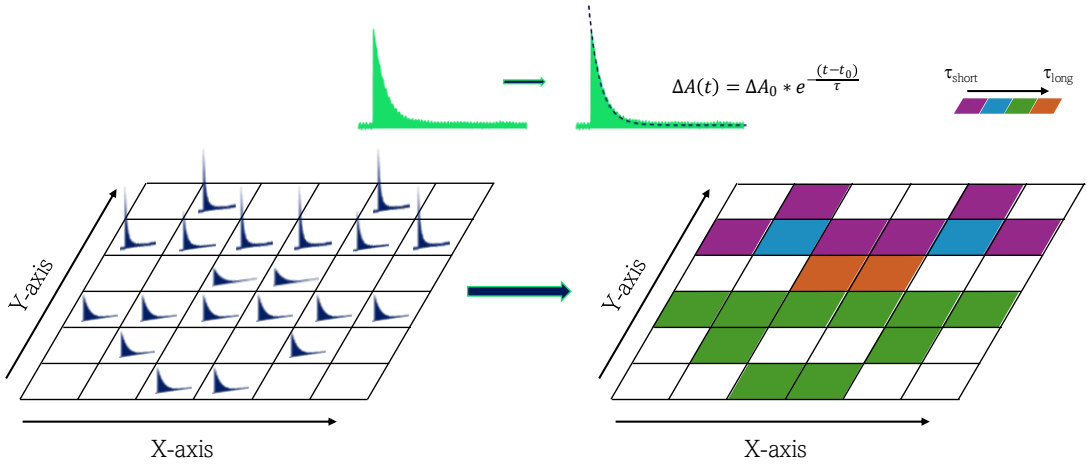
**Keywords:** Time-Resolved Absorbance; Imaging

Laser flash photolysis [1][2] (LFP) has been a fundamental technique for investigating fast photochemical processes by capturing transient absorption spectra. Traditionally, it has been a one-dimensional method, measuring absorption changes at a single spatial point. However, expanding this method into a 2D imaging system enhances its analytical power by mapping spatial variations in transient absorption across a sample.




Inspired by Fluorescence Lifetime Imaging Microscopy [3][4] (FLIM), which provides spatial resolution in the traditional time-resolved fluorescence measurements, this study presents the development of a Transient Absorption Imaging System (TAIS). TAIS expands upon conventional LFP by generating a matrix of transient absorption spectra, where both lifetime ($\tau$) and amplitude ($\Delta A_0$) values are mapped into corresponding images. By integrating a 2D approach, TAIS opens new possibilities for studying heterogeneous photochemical environments with improved spatial resolution. The initial validation of the system was conducted using microfluidic slides containing well-characterized dye solutions as samples. Our current setup features a photodiode detector alongside a dual-laser configuration: a high-frequency pulsed laser serving as the pump excitation source and a continuous-wave (CW) laser for probe monitoring. By employing a CW laser as source instead of the conventional white lamp used in traditional LFP, we eliminated the need for a monochromator, resulting in a significantly more compact and efficient system. For spatial imaging, a manual 2D translation stage is currently implemented to facilitate scanning acquisition. Preliminary results are highly promising, demonstrating the system's capability to capture spatially resolved lifetime variations with a spatial resolution under 50µm.

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# Cluster triggered emission (CTE) materials applied to antimicrobial photodynamic therapy (aPDT)

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**Keywords:** Photosensitizers (PS); antimicrobial Photodynamic Therapy (aPDT); Cluster Triggered Emission (CTE)

Antibiotic resistance threatens global health, with projections estimating 8.22 million deaths annually by 2050[1]. Conventional treatments struggle as bacteria continuously mutate, making antibiotics less effective.

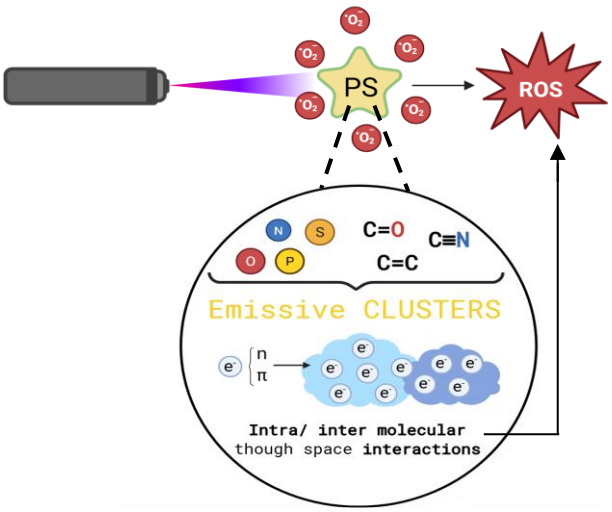


**Figure 1. Graphical abstract. Created with Biorender.**

Antimicrobial photodynamic therapy (aPDT) offers a promising alternative, using a harmless light source and photosensitizer (PS) to generate cytotoxic singlet oxygen[2]. However, developing efficient, biocompatible, and stable PS molecules remains a challenge. Early PS compounds suffered from aggregation-related quenching, leading to the exploration of aggregation-induced emission (AIE) materials[3]. While AIE compounds improved emission, they lacked solubility and biocompatibility, prompting the development of third-generation PS[4].

These newer PS materials feature heteroatoms and unsaturated bonds, forming emissive clusters known as cluster-triggered emission (CTE) materials[5]. Their electron cloud interactions facilitate light absorption, emission, and singlet oxygen production.

Commercial CTE materials have been tested for key photochemical properties, with the most promising candidates undergoing microbiological photoinactivation, showing encouraging initial results. These advancements reinforce CTE materials as strong contenders for aPDT, offering hope against bacterial resistance.

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# Push-pull phenalenones for oxygen-independent PDT

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**Keywords:** Photoredox catalysis, reactive oxygen species, hydroxyl radicals, oxygen-independent

PDT is a promising light-activated cancer treatment that generates reactive oxygen species to destroy malignant cells. [1,2] However, its clinical use faces significant obstacles: limited light penetration into tissues, poor water solubility of many photosensitizers, and crucially, the hypoxic environment found in solid tumors, which severely hinders ROS production. This research introduces a new strategy to overcome tumor hypoxia generating cytotoxic hydroxyl radicals directly from water through photocatalytic oxidation. [3] We're focusing on 6-amino-1H-phenalen-1-one (6-APN) [4], a photosensitizer our group has previously studied. Earlier research showed that 6-APN produces ·OH even without oxygen, via a light-induced intramolecular electron transfer that oxidizes water. Beyond 6-APN known ability to generate singlet oxygen[5] in traditional PDT, this study also explores its potential in photoredox catalysis. By leveraging 6-APN unique electron donor-acceptor structure, we aim to demonstrate its capacity to drive robust ROS generation directly from water. This innovative approach seeks to significantly enhance PDT's effectiveness in challenging hypoxic tumor environments, ultimately unlocking its full clinical potential.

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**Photodynamic Therapy and Diagnosis**

# PHOTOSTABILITY OF DURVALUMAB (IMFINZI®) DURING ROUTINE HOSPITAL HANDLING: EFFECTS ON BIOLOGICAL ACTIVITY AND IMMUNOGENICITY

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**Keywords:** Monoclonal Antibody; Photostability; Immunogenicity; Biological Activity.

## ABSTRACT

The use of monoclonal antibodies (mAbs) in pharmacological therapies has greatly increased, supported by progresses in research and technologies that enhance their specificity and stability. However, as protein-based drugs, mAbs are highly sensitive to degradation, making strict storage and handling conditions essential, as outlined in the Summary of Product Characteristics (SmPC). In hospital settings, mAbs are diluted before administration for intravenous infusion, exposing them to various physical and environmental stresses such as temperature fluctuations, agitation, and light. These factors may potentially affect the drug's stability and therapeutic efficacy. [1]

This study investigated the effects of simulated indoor sunlight exposure on the monoclonal antibody Durvalumab (Imfinzi®), which is approved for the treatment of several solid tumours. The antibody was diluted in saline solution at the clinically use concentrations, 1mg/mL and 15 mg/mL, and exposed to a light dose equivalent to approximately 8-10 hours of sunlight. Its secondary and tertiary structures, along with physico-chemical modifications and biological activity, were assessed using spectroscopic techniques, SDS-PAGE, immunogenicity tests and cell-based bioassays.

Results showed that Durvalumab's secondary and tertiary structures remained stable upon light exposure. However, a decrease in target recognition (PD-L1) was observed in cellular assays, while the formation of antibody aggregates was minimal and did not appear to induce immunogenicity.

In conclusion, light exposure during hospital preparation may slightly reduce the biological activity of Durvalumab, potentially impacting its antitumor efficacy. Therefore, protecting the drug from sunlight or artificial light during handling and administration could help preserving its therapeutic effectiveness.

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**Photodynamic Therapy and Diagnosis**

# *In vitro* effectiveness of novel phthalocyanine-based photosensitizers in photochemical internalization of saporin

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**Keywords:** PCI, phthalocyanine, saporin

Development of approaches for specific drug delivery is important in the therapy of cancer and other diseases as well. The use of biologically active macromolecules has become very important in the treatment of various diseases. However, most of these macromolecules enter the cells by endocytosis and molecules taken up in this way are usually unable to leave endosomes. Subsequently they are degraded in lysosomes and lose their therapeutic effect. In many cases, internalization into cytosol is the key factor to achieve the expected effect (1) (2). Photochemical internalization (PCI) is one way to release these macromolecules into cytosol. PCI is based on principles shared with photodynamic therapy. However, the photosensitizers (PSs) in PCI are used only to damage endosomal membranes and release the cargo into the cytosol where it can affect its target. PCI represents a method for selective drug delivery to cancer cells, which can increase their effect. Hence, the adverse effects of the drug may be lower due to decreased dosage (3)(4). The aim of this project is to identify novel phthalocyanine PSs suitable for PCI. PSs used in our study are differently modified amphiphilic charged phthalocyanines. Human cervical carcinoma cell line (HeLa) was used for all experiments. At first, the inherent cytotoxicity of saporin and phototoxicity of PSs were evaluated and their $IC_{50}$ values were determined. All experiments were done in both „light after“ and “light before” approaches with 4 and 18 h incubation followed by 4 h chasing period. Irradiation was performed using 450 W Xe lamp (15 min, $\lambda > 570$ nm, 11.2 J/cm$^2$). Cell viability was evaluated 24 h post irradiation by neutral red uptake assay. The $IC_{50}$ values were used to generate a concentration series corresponding to multiples and fractions of their $IC_{50}$ values (/8, /4, /2, ×1, ×2 and ×4). Cytotoxicity of PSs, saporin and their combinations was evaluated and combination indexes were determined using the Chou-Talalay method. In most cases, strong synergism effect was found.

**Acknowledgments:** This project was funded by Charles University Grant Agency grant no. 1312220.

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# "Nanoscintillators: enabling deep-tissue photodynamic therapy and enhancing radiotherapy on glioblastoma multiforme"

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**Keywords:** Nanoscintillators, Radiotherapy, Photodynamic Therapy, Glioblastoma multiforme, Cancer

Glioblastoma (GBM) is the most aggressive primary brain tumor in adults, with a median survival of 12–15 months. Despite surgery, radiotherapy (RT), and temozolomide, which are the current standard of care, prospects for patients have remained virtually unchanged over the past two decades [1]. As such, there is a pressing need for innovative treatments for GBM.

We studied the use of nanoscintillators as next-generation radiotherapeutics, particularly inorganic crystals doped with rare earth elements. Upon interaction with ionising radiation, such as X-rays used for RT these particles offer a dual physical advantage: (i) Radiation dose enhancement (RDE): High atomic number (Z) materials have a greater probability of absorbing X-rays, thereby increasing local radiation dose deposition around the nanoparticle. (ii) X-PDT: photons in the visible range produced during scintillation can activate light-based therapies, namely, Photodynamic therapy (PDT). PDT relies on light, molecular oxygen, and a photosensitizer molecule (PS) to generate reactive oxygen species (ROS). The combination of PDT and RT is interesting because they both lead to cellular death in mechanistically distinct ways, with some studies reporting a synergistic effect [2][3]. Typically, PDT is limited by the penetration depth of light, however, the use of nanoscintillators acting as a local light source could enable this therapy for GBM during RT.

Grafting or loading PSs to the surface of nanoscintillators yields promising results [4], however, this conjugation represents a longer synthesis. We studied an innovative approach, which leverages a clinically approved, endogenously produced PS called PpIX, which selectively accumulates inside cancer cells after its precursor 5-ALA is injected into patients intravenously [5], and tumor-accumulated nanoscintillators. We investigated the ability of nanoscintillators to efficiently excite PpIX upon X-ray irradiation and generate reactive oxygen species in solutions and in vitro. We aim to unravel the mechanisms triggered during RT.

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**Photodynamic Therapy and Diagnosis**

# 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

Autophagy is a crucial quality control mechanism that degrades damaged cellular components through lysosomal fusion with autophagosomes. However, elevated autophagy levels can promote drug resistance in cancer cells, enhancing their survival. Downregulation of autophagy through oxidative stress is a clinically promising strategy to counteract drug resistance, yet precise control of oxidative stress in autophagic proteins remains challenging. Here, we demonstrate a molecular design strategy of biocompatible neutral Ir(III) photosensitizers, B2 and B4, for precise reactive oxygen species (ROS) control at lysosomes to inhibit autophagy. We explore the underlying molecular mechanisms for the biocompatibility and lysosome selectivity of Ir(III) complexes by comparing B2 with the cationic or the non-lysosome-targeting analogs. Also, the biological mechanisms for autophagy inhibition via lysosomal oxidation are explored. Proteome analyses reveal significant oxidation of proteins essential for autophagy, including lysosomal and fusion-mediator proteins. These findings are verified in vitro, using mass spectrometry, live cell imaging, and a model SNARE complex. The anti-tumor efficacy of the precise lysosomal oxidation strategy is further validated in vivo with B4, engineered for red light absorbance. This study is expected to inspire therapeutic use of spatiotemporal ROS control for treating autophagy-derived resistant cancers.

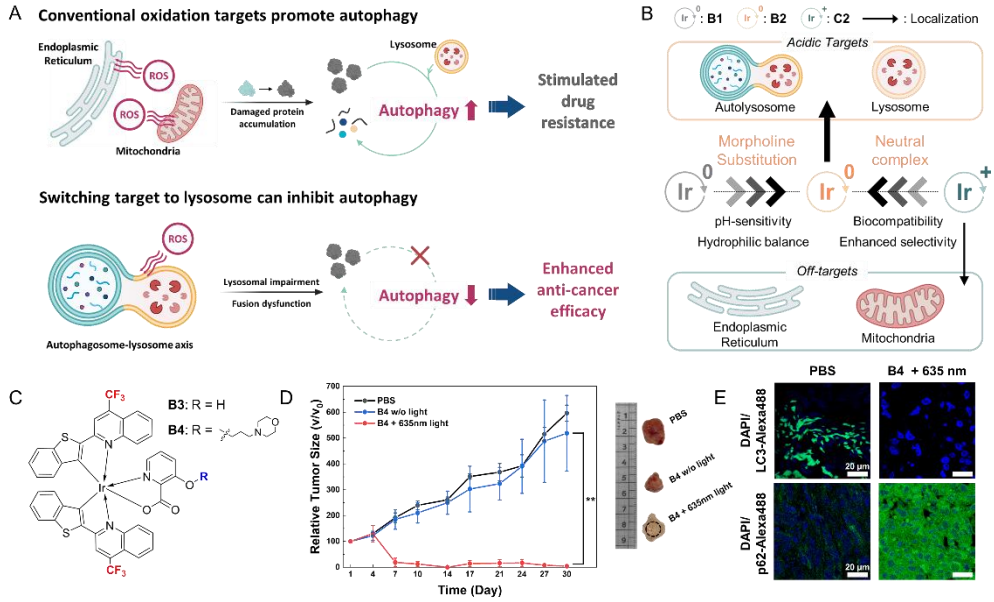


**Fig. 2.** (A) Schematic illustration of the dual role of reactive oxygen species (ROS) in autophagy. Oxidative stress can modulate autophagy in either a stimulatory or inhibitory manner. (B) Summary of molecular mechanism for lysosome-specific biocompatible neutral Ir(III) complex with morpholine substitution. (C) Molecular structure of our therapeutic lead compound, B4. (D) The change of tumor size ($v/v_0$) in intrinsically drug-resistant cell line, Panc-1, for 30 days shown with the representative ex vivo tumor images after sacrifice. (E) LC3 and p62 expression levels (green) in tumor tissues examined by immunohistochemical staining with or without B4 photosensitization (Blue: DAPI, Scale bar = 20 μm).

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**Photodynamic Therapy and Diagnosis**

# Designing Polymer-Based Delivery Systems for Hydrophobic Phthalocyanine Photosensitizers: Impact of Hydrophilic-Hydrophobic Balance in Polymer on Photobiological Outcomes

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**Keywords:** photodynamic inactivation; phthalocyanines; antibacterial polymers; drug delivery.

The growing threat of antibiotic resistance and the persistent challenge of biofilm-associated infections demand alternative therapeutic strategies. Biofilms are protective bacterial communities embedded in an extracellular matrix, significantly reduce the efficacy of conventional antibiotics[1]. Innovative drug delivery systems are therefore essential to enhance antimicrobial penetration and effectiveness[2]. Hydrophobic phthalocyanines (Pcs) are potent photosensitizers for antimicrobial photodynamic therapy (aPDT), but their poor aqueous solubility limits biological application. In this study, we developed six polymeric micellar systems to encapsulate zinc phthalocyanines with either phenyl (Ph) or pyridine (Py) substitutions. The polymers consisted of a fixed hydrophobic block and variable hydrophilic block lengths, modulating their hydrophilic-hydrophobic balance. Successful Pc loading was confirmed by UV-Vis spectroscopy, while micelle size and morphology were analyzed using DLS and SEM. Pc-loaded micelles, especially those with long hydrophilic chains, showed enhanced antibacterial and antibiofilm activity under light activation, with performance influenced by Pc substitution pattern and the local biofilm microenvironment.

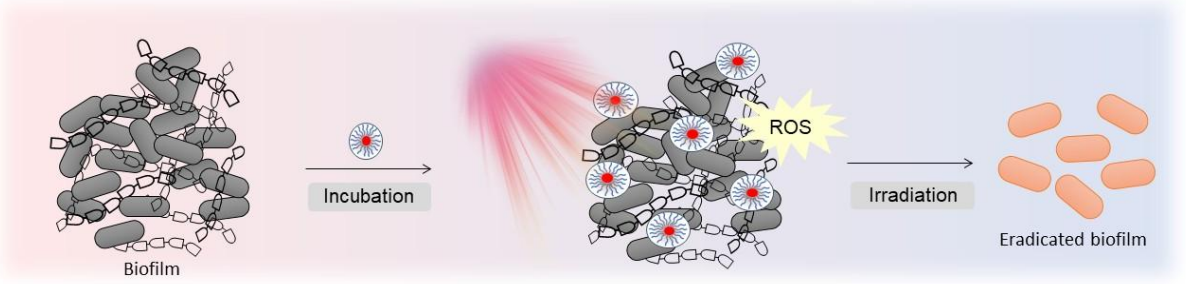


***Figure 1**. Schematic illustration of the general strategy of the project.*

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# Porphyrins with *N*-oxide moiety: synthesis, (photo)physical characterization and *in vitro* study of PDT on melanoma cell lines in hypoxic conditions

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**Keywords:** amphiphilic porphyrins; photodynamic therapy; *N*-oxide moiety; hypoxia

Hypoxia in solid tumor microenvironments is one of the main obstacles in the effectiveness of photodynamic therapy (PDT), as it limits available oxygen needed for generating cytotoxic reactive oxygen species and is responsible for promoting the tumour resistance by increasing the angiogenesis, invasion and metastasis [1]. Thus, there is an urgent need for new photosensitizes (PSs) to overcome these limitations. Porphyrins with *N*-oxide substituents in the structure, as a moiety of hypoxia-activated prodrugs (HAPs,) are tested as a strategy to overcome hypoxia and potentially activate other cytotoxic mechanisms. HAPs activated by hypoxia undergo a reduction by two electrons leading to the formation of free radicals and/or active molecules that can then react with DNA or proteins [2]. Our group recently showed that amphiphilic *N*-methylated pyridiniumporphyrins with a longer alkyl chain (>13C atoms) are efficient PSs against melanoma cell lines, due to their increased cellular uptake [3]. In addition, porphyrin with 13C atoms-long chain showed increased selectivity towards melanoma cell lines [3].

Here we present our study of a group of porphyrins bearing an alkyl chain of different lengths (13C and 17C atoms) with *N*-oxidized pyrid-3-yl groups for PDT. Their properties are evaluated by absorption and fluorescence spectroscopy, laser pulse photolysis (LFP), time-correlated single photon counting (TC-SPC) and by determination of singlet oxygen production (DPBF photodegradation) and the lipophilicity of the molecules. The impact of the chain length was investigated *in vitro* by analyzing cellular uptake, localization of PS and (photo)cytotoxicity in different melanoma cell lines (B16F10, MeWo and A375) and fibroblasts under conditions of normoxia and hypoxia, induced by $CoCl_2$ hypoxia mimetic, GOX/CAT enzymatic system or use of oxygen scavengers. The results are compared with hydrophilic analogue with acetamido group and with *N*-methylated analogues with the same alkyl chain length.

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# Light-Directed Activation of a PD-L1-Targeting Immunotoxin for Selective Elimination of Immunosuppressive Tumor Cells

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**Keywords:** Immunotoxins; Targeted Cancer Therapy; Photochemical Internalization; Recombinantly Produced Therapeutics

Immune checkpoint inhibitors (ICIs) against the PD-1/PD-L1 axis show significant clinical success in some cancers such as melanoma, by restoring cytotoxic T-cell responses. However, their administration is associated with immune-related adverse events and limited response. These challenges can be met with immunotoxins (ITs) utilizing antibodies targeting the PD-1/PD-L1 axis for the delivery of toxins. Cancer specificity of the treatment can be further increased by the use of toxins which are entrapped within the endo/lysosomal system and therefore are dependent on site-specific endosomal destabilization in order to exert efficacy. In this project, we address these limitations by developing a novel recombinant immunotoxin, αPD-L1-rGel (PG100), designed for light-induced intracellular activation by photochemical internalization (PCI). PG100 was recombinantly expressed in *Escherichia coli*, following molecular cloning. Purification of the targeted toxin was performed with the ÄKTA Avant™ automated liquid chromatography system in sequential steps. Initial characterization will include assessment of PD-L1-binding as well as *in vitro* cytotoxicity studies in human and murine cancer cell lines with varying PD-L1 expression, both with and without PCI. Further optimization of the targeted toxin sequence and purification method will be performed before PG100 will be tested *in vivo* in both immunodeficient and immunocompetent murine tumor models. By combining the target specificity of a checkpoint inhibitor, the potent cytotoxic potential of gelonin, and the site-specific activation mediated by PCI, this approach should offer a safer and more controllable alternative for selective elimination of PD-L1-expressing tumor cells than currently available treatment.

# 5-ALA Mediated Radiodynamic Therapy Using Gold Gold Sulfides

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**Keywords:** Radiodynamic therapy, 5-aminolevulinic acid, gold gold sulfide.

## ABSTRACT

Radiotherapy (RT) is an effective medical treatment that eliminates cancer cells [1]. While ionizing radiation successfully targets tumor areas, it may also damage surrounding healthy tissue. Radiodynamic therapy (RDT) is a promising modality that enhances the effects of radiotherapy and increases the potential of photosensitizers, benefiting from the limitless penetration depth of X-rays [2]. 5-Aminolevulinic acid (5-ALA), a precursor of the photodynamic therapy agent protoporphyrin IX (PpIX), is commonly used for local treatments. Elements with high atomic numbers, such as gold, have strong potential for radiosensitization. Our research group successfully synthesized gold–gold sulfide (GGS) nanoparticles, which show great promise as radiosensitizers [3]. In this study, we combined GGS and 5-ALA using electrostatic loading to utilize radiotherapeutic and photodynamic effects through RDT. Although 5-ALA is commercially available and frequently used as a photosensitizer, its application is limited due to low bioavailability and low penetration depth of phototherapy. GGS acts as a radiosensitizer by absorbing X-rays and can enhance reactive oxygen species (ROS) generation by transferring energy to the photosensitizer. Gold–gold sulfide nanoparticles were synthesized in an aqueous environment, and 5-ALA was loaded onto the GGS particles. PC-3 and MDA-MB-231 cells were used for in vitro experiments. Cell viability was evaluated using the MTT assay. Radiation treatment was performed using a self-contained X-ray cabinet with a 4 Gy dose. Electrostatic loading of 5-ALA onto 3-MPA-coated GGS nanoparticles was successfully demonstrated using isothermal titration calorimetry (ITC). Cytotoxicity experiments using the PC-3 cell line showed no significant toxicity without radiation after 24 hours of incubation at 25–100 µg/mL Au concentration. GGS nanoparticles, 5-ALA alone, and 5-ALA-loaded GGS particles were treated with radiation. Enhanced damage was observed in the GGS–ALA group following 4 Gy radiation. In conclusion, 5-ALA was successfully loaded onto GGS nanoparticles, enhancing their theranostic potential through radiodynamic therapy.

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# Photodynamic inactivation of *Escherichia coli* and *Staphylococcus aureus* by cationic diketopyrrolopyrroles

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**Keywords:** Diketopyrrolopyrrole; Photodynamic inactivation; Reactive oxygen species (ROS); Reactive iodine species (RIS).

Diketopyrrolopyrroles (DPP) are a family of organic fluorophores characterized by an exceptional stability and outstanding photophysical and electronic properties.[1] These features have made them especially attractive as colorants in prints and inks or as active materials in optoelectronic devices, including organic light-emitting diodes (OLEDs), organic field-effect transistors (OFETs), and organic photovoltaic (OPV) cells.[1,2] More recently, DPP gained special relevance for biological applications, including for bioimaging, or as photosensitizers for photothermal therapy (PTT), photodynamic therapy (PDT) or as theranostic agents.[3-5]

In this work, the potential of two new mono and dicationic diketopyrrolopyrroles to photoinactivate Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* was investigated under white light irradiation (50 mW $cm^{-2}$). Despite the low production of singlet oxygen (< 4%), these compounds were able to effectively inactivate the bacteria when combined with potassium iodide (KI), an effect probably due to the production of reactive iodine species (RIS). In brief, our results show that, at a concentration of 5.0 μM, the dicationic compound (+ KI) photoinactivated *S. aureus* to the detection limit of the method (7.00 $\log_{10}$ CFU reduction) in only 5 min. Under similar conditions, it photoinactivated the bioluminescent *E. coli* to the detection limit (4.83 $\log_{10}$ RLU reduction) in 10 min of irradiation.

Given the limited number of studies addressing the photodynamic inactivation of microorganisms using DPP derivatives, our findings highlight the potential of these compounds for the photodynamic treatment of bacterial infections.

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# Development of Near-infrared Fluorescent and Photoacoustic Thiadiazole Quinoxalines

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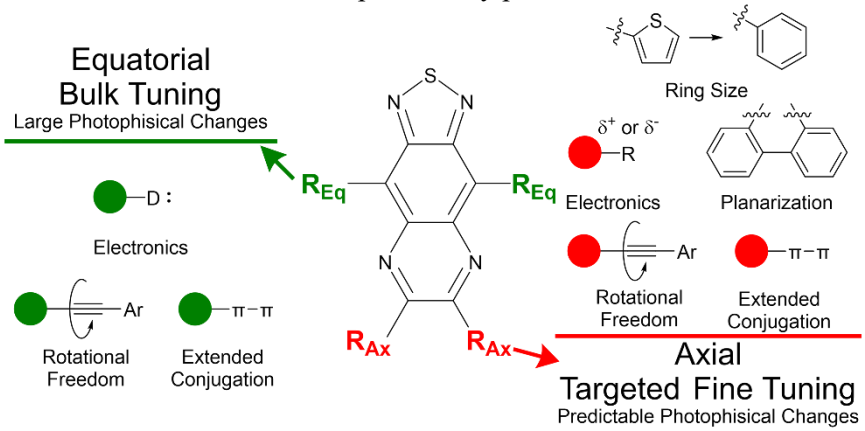
**Keywords:** Photomedicine; Imaging; Fluorescence

Near infrared (NIR) chromophores have garnered significant attention for biological applications[1,2] due to the deceased absorption of NIR light by biological molecules and decreased scatter compared to visible light, leading to deeper penetration of excitation light through tissue.[3] Thus exogenous NIR chromophores can be used for biological imaging *in vivo*.[2]
Thiadiazole quinoxaline (TQ) derivatives have been reported with NIR absorption with potential in both fluorescence [4] and photoacoustic imaging.[5] The photoacoustic effect refers to the process of light to sound conversion. Following absorption of ns-pulses of light, non-radiative decay leads to heating and an acoustic wave is then produced via a local pressure change.[6] Therefore by control of the radiative and non-radiative processes, a probe can be optimally designed for fluorescence or photoacoustic imaging. By synthesizing and comparing a large library of TQs, we observe that the choice of 'axial' and 'equatorial' substituents can controllably tune their photophysical properties such as their absorption spectra and radiative- and non-radiative rates, making TQs potential biological probe scaffolds that can be readily modified. Photoluminescence and preliminary photoacoustic data will be discussed.

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# Porphyrin-triphenylphosphonium conjugate: an efficient photosensitizer for photodynamic therapy in Panc-1 cells

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**Keywords:** cationic porphyrins; pancreatic cancer; reactive oxygen species; apoptosis

Pancreatic cancer is among the most lethal malignancies, primarily due to its late diagnosis and limited response to conventional therapies [1]. Photodynamic therapy (PDT) offers a minimally invasive and tumor-selective alternative, relying on the activation of a photosensitizer (PS) by light to generate reactive oxygen species (ROS) that induce localized cell death [2]. Recent clinical studies have highlighted the potential of PDT in the treatment of pancreatic cancer [3]. Among the available PSs, cationic porphyrins have attracted attention due to their enhanced phototoxicity. In this context, the aim of this study was to investigate the efficacy of a highly charged octacationic porphyrin conjugate bearing triphenylphosphonium units (Por-$PPh_3$) [4], as a PS for PDT in Panc-1 pancreatic cancer cells. Cells were treated with Por-$PPh_3$ (0.005-10 μM) for 24, 48 and 72 h, and cell viability was assessed by MTT assay. A slight decrease in viability was observed at concentrations ≥ 0.25 μM after 48 and 72 h, with the most pronounced effect seen at 10 μM after 72 h. To assess photodynamic effects, cells were exposed to Por-$PPh_3$ (0.005–0.25 μM) for 24 h for internalization, and the plates were exposed to visible light using a LED (2.5 and 5 J/cm$^2$) or kept in the dark. Cell viability was evaluated after 2, 24 and 48 h. After PDT treatment, a marked decrease in cell viability was observed after only 2 h post-PDT at concentrations ranging from 0.05 to 0.25 μM; this reduction persisted at 48 h post-PDT. A comparison of light doses revealed that 5 J/cm$^2$ had a greater impact in cell viability than 2.5 J/cm$^2$ at 0.05 and 0.1 μM, but the results were similar at 0.25 μM. The assessment of intracellular ROS levels measured at 0.05 and 0.1 μM, showed a significant increase at both 2 h and 48 h post-PDT, particularly at the later time point. Flow cytometry analysis further revealed that, at the same concentrations, under 5 J/cm² irradiation, Por-$PPh_3$ promoted early apoptosis. In this communication, full details and discussion will be presented confirming that Por-$PPh_3$ is a promising PS candidate for PDT in pancreatic cancer, effectively decreasing cell viability by increasing ROS levels and leading to apoptosis.

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# Photodynamic therapy utilizing porphyrin lipid nanoparticles: results from a veterinary study in naturally occurring oral cancers in cats and dogs

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**Keywords:** Oral Cancer; photodynamic therapy; veterinary; fluorescence imaging.

## EXTENDED ABSTRACT

**Introduction.** Oral cancers are challenging to treat with a poor prognosis in many veterinary patients. Cats in particular respond poorly to the current standard of care treatments and are observed to have high rates of treatment failure. Porphysomes (pPs) are porphyrin-lipid based nanoparticles with multifunctional capabilities, including dual utility as a fluorescence imaging agent and a photodynamic therapy (PDT) photosensitizer. Utilizing near-infrared fluorescence (NIRF), pPs can provide intraoperative information regarding tumor delineation and facilitate fluorescence guided surgery (FGS) through tumor margin identification. pPs-PDT has been extensively tested in preclinical mouse models of oral cavity squamous cell carcinoma (OCSCC), showing compelling results when pPS-PDT is combined with surgery. To continue to investigate pPS PDT and FGS for oral cancer treatment, a collaborative veterinary clinical trial in companion dogs and cats with spontaneous oral cancers was started, with safety, feasibility, and therapeutic efficacy assessments as primary aims. This trial serves as a valuable intermediary between studies in preclinical rodent models and human studies.
**Methods and materials**. Three dog and three cat (all SCC) patients with tongue tumors received 3mg/kg pPs doses via slow infusion. At 24 h post-injection, in vivo tissue concentrations of pPS were quantified using diffuse reflectance imaging and fluorescence spectroscopy as previously developed. Tumors were then irradiated with a light dose of 12.5-75 J/cm$^2$ from a 671 nm diode laser using interstitial (in the case of bulky tumors > 8mm deep) or external fibers (in the case of superficial tumors <8mm deep). Resected or biopsied specimens were processed for histologic analysis (H&E, PanCK, TUNEL assay) to evaluate treatment response. Participants were followed up at 2 weeks, 1 month and as required clinically thereafter. Treatment response was assessed clinically and by CT/ultrasound or MRI. Standard quality-of-life measurements were made during all veterinary evaluations throughout the study. End point variables included histology analysis for the 'pPS ablate and surgically resect' section, disease free survival and overall survival for adjuvant and monotherapy pPS-PDT, and response evaluation criteria in solid tumors criteria in palliative treatments.
**Results.** No adverse reactions to the infusion of pPS and/or during PDT were observed. Post-procedure complications were observed in 2 animals, were self-limiting and required supportive care, fluids, and overnight hospitalization. One cat experienced distal necrosis of the tongue and underwent debridement of the devascularized region. Overall, all animals' quality of life after the procedure improved. Measurable tumor response following PDT was noted in 83% of the cohort. Two animals (one dog and one cat) are alive and doing well 48- and 52-weeks post-treatment, including one cat treated with monotherapy PDT with negative biopsies obtained 4 months post treatment.
**Conclusions.** Tongue tumors are challenging to treat with a poor prognosis in cats and dogs. This is the first in-patient study of pPS-PDT in a naturally occurring oral cancer, with initial data demonstrating safety and therapeutic efficacy. Additionally, this represents a high-fidelity clinical setting that provides unparallel translational research knowledge (techniques, equipment and workflow) that serves as a developmental intermediary for OCSCC human clinical trials.

**Photodynamic Therapy and Diagnosis**

# Nanoparticle-mediated Photo-Chemical Immune Stimulation in neoadjuvant treatment of pre-clinical oral cavity cancer models

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**Keywords:** Oral Cancer; photodynamic therapy; neoadjuvant treatment, immune system

***Introduction***. Photodynamic therapy (PDT) is a nonsurgical and tissue sparing ablation technique, able to selectively target tumour cells by activating a photosensitizer with light to produce well localized cytotoxicity. We previously reported development of porphysome (PS), porphyrin-lipid nanoparticles capable of potent PDT in multiple tumor models. As well as destroying the targeted tumor tissue, recent evidence suggest that PS-based PDT can stimulate a systemic anti-tumor immune response. Oral cavity squamous cell carcinoma (OCSCC) represents the most prevalent form of head and neck cancer, with a risk of local or local-regional recurrence following surgical treatment that increases with stage of disease, ranging from 15 to 60%. The present study is intended to explore Photo-Chemical Immune Stimulation (PCIS) in preclinical OCSCC models when treated with PS-based PDT in the neoadjuvant setting.

***Methods and materials***. A group of 30 mice was employed. Mouse oral squamous cell carcinoma (MOC2) cells were injected subcutaneously in the right flank in immune competent 8–10 weeks old female C57BL/6J mice. After 7 days, PS nanoparticles were intravenously administered to MOC2 bearing mice at a dose of 10 mg/kg and mice were then randomized in 2 groups: (1) the control group, where mice (nr 15) had surgery only (day 10 after tumour injection); (2) the treatment group, where mice (nr 15) underwent PDT (35J) twenty-four hours post-PS injection, followed by surgery after 48 hours (day 10 after tumour injection). Surgery was standardized for each animal, performing a tumor gross resection with intentional microscopic residual disease, proven by histological analysis. Time to tumour recurrence and volume growth rate were determined over a 2 month time follow-up. Also, a luminol assay was performed after PDT treatment in 2 different timepoints, 1hr and 24hrs after PDT.

***Results.*** Time to recurrence was delayed in the PDT treatment group, with only 30% of cases presenting tumor recurrence. In the control group, all animals presented obvious disease within 12 days after surgery. In the control group, the tumor volume regrowth was significantly higher in comparison to the treatment group, where tumor volume, when recurrence occurred, remained stable over time.

A luminol assay was performed in both groups, 1 hr and 24 hrs after PDT, showing an increased neutrophilic activity in the PDT treatment group when compared to the control group.

***Conclusions.*** Our preliminary results in preclinical OCSCC models shows evidence of tumor volume re-growth control after PS-PDT treatment, suggesting the existence of photo-chemically induced immune system activation. Further experiments are currently ongoing to quantify and optimize the PCIS effect on tumor progression. Effective development of PDT mediated PCIS would be beneficial in patient populations at risk of local recurrence and regional progression.

# BODIPY complexes conjugated with nucleosides and sterols for antimicrobial photodynamic therapy

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**Keywords:** heavy-atom free photosensitizers, antimicrobial resistance, photodynamic therapy

Antimicrobial resistance remains a major global health challenge and continues to represent a significant cause of death worldwide [1]. Photodynamic therapy is an alternative strategy for the treatment of bacterial and fungal infections [2]. The therapy involves the administration of the photosensitizer, that localized in the microbial cells, followed by targeted irradiation with a light source. Upon activation, the photosensitizer undergoes transition to triplet excited states *via* intersystem crossing (ISC), and interacts with molecular oxygen present in the cells, resulting in reactive oxygen species (ROS) generation and subsequent cell death. This mechanism hinders the development of resistance due to its universal nature. A photosensitizer and its ability to generate ROS are important to ensure the efficacy of therapy. Therefore, the development of novel, highly effective, and selective photosensitizers with minimal dark cytotoxicity remains crucial.

As potential photosensitizers, BODIPY dyes are intensively studied due to efficient absorption in the visible light region, feasible synthesis, and high structural tunability [3]. The straightforward and highly efficient strategy to enhance ISC and excited triplet state generation is the incorporation of heavy atoms into the structure of photosensitizers [4]. Nevertheless, these systems are often characterized by significant dark cytotoxicity and poor water solubility, which hampers their application in PDT. Hence, the heavy-atom free photosensitizers are intensively studied. The common approach to increase ISC yields in these systems is the employment of donor-acceptor molecular architectures that provide nearly orthogonal alignment. Upon light irradiation, such architecture enables the intramolecular photoinduced electron transfer, resulting in the formation of an intermediate excited charge transfer state and subsequent ISC to the triplet state [5].

Herein, we present a series of novel, highly photoactive BODIPY complexes featuring an organoboron scaffold conjugated with biomolecules, such as nucleosides and sterols, for application in antimicrobial photodynamic therapy. The partial involvement of the boracyclic moiety in triplet state generation enabled further structural modifications at positions inaccessible in previously reported heavy-atom free BODIPY dyes with donor-acceptor architecture. This rational design allowed for the enhancement of cellular uptake and selectivity towards microbial cells, without affecting the photocatalytic properties.

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**Photodynamic Therapy and Diagnosis**

# Aloe emodin-mediated antimicrobial photodynamic therapy inactivates Candida auris biofilm using visible light

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**Keywords:** Aloe vera; visible light; super fungus; aPDT.

*C. auris*, an emerging multidrug-resistant fungal pathogen, poses a significant health risk with high mortality in systemic infections and exhibits resistance to multiple antifungal classes, alongside virulence factors like biofilm formation [1]. Antimicrobial photodynamic therapy (aPDT) presents a promising alternative due to its non-invasiveness and lack of resistance induction, using photosensitizers (PS) activated by light to generate reactive oxygen species [2]. Natural polyphenolic compounds, such as *Aloe emodin* (AE), a natural anthraquinone derivative with various pharmacological properties, are being explored as PS in aPDT [3]. In this way, this study explored the in vitro potential of AE-mediated aPDT against the emerging multidrug-resistant fungus *C. auris*. Antimicrobial activity of AE was analyzed against other multidrug-resistant bacterial pathogens like *Staphylococcus aureus* NTCC 12493, *S. aureus* ATCC 25923, *Escherichia coli* ATCC 13486, and E. coli ATCC 25922, as well as *C. auris* CDC B11903, by the disk-diffusion method. Also, molecular docking simulations for AE and the apo crystal structure of *C. auris* dihydrofolate reductase (DHFR) were also realized for analyses of binding affinity. aPDT for *C. auris* involved a $1 \times 10^7$ CFU $mL^{-1}$ suspension incubated with 10 μM of AE for 30 min in the dark, followed by irradiation with 400-780 nm light (25 mW $cm^{-2}$, 96 J $cm^{-2}$) for 15 min, with colony counts on PCA plates. *C. auris* biofilm inhibition was assessed using a 24-hour culture in 96-well plates with AE serial dilutions (1000-0.01 μM), quantified by crystal violet staining. *C. auris* biofilm eradication involved forming a 48-hour biofilm, followed by AE treatment (1000-0.01 μM) for 16-20 hours and crystal violet quantification [12]. Statistical analysis employed two-way ANOVA with Tukey's post hoc test. While AE alone showed no significant antimicrobial nor antifungal activity, its activation with visible light (400-780 nm) at 10 μM resulted in a complete (100%) reduction of viable *C. auris* colony counts after 15 minutes of exposure. Conversely, the dark control group exhibited only a 30% decrease. AE also significantly inhibited *C. auris* biofilm formation from 1 μM concentration under light, being concentration dependent. AE-aPDT was less effective against established biofilms, suggesting a greater impact on biofilm development than on mature structures. Furthermore, AE-aPDT demonstrated a notable capacity to inhibit the initial development of *C. auris* biofilms. AE also exhibited a stronger binding affinity toward the DHFR enzyme of *C. auris*, with a binding energy of −7.8 kcal/mol, a ligand efficiency (LE) of 0.390, a binding efficiency index (BEI) of 0.029, and an estimated inhibition constant (Ki) of 1.901 μM. This study underscores the significant potential of visible light-activated AE-mediated aPDT for inhibiting *C. auris* biofilm formation and eradicating planktonic cells, emphasizing the critical role of light as an environmental factor in disinfection efficacy. The rapid and complete eradication of *C. auris* under these conditions suggests a promising avenue for developing disinfection protocols and potential therapeutic interventions against this critical global health threat.

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# Influence of methylene blue formulations on the efficacy of photodynamic inactivation of *Candida albicans*

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**Keywords:** Light-based technology; Antifungal resistance; Antimicrobial Photodynamic therapy

The fungal resistance observed in the current clinical scenario has intensified the need for innovative and effective therapeutic alternatives. In this context, photodynamic therapy has emerged as a promising strategy; so far, it does not appear to induce microbial resistance. This study evaluated the effectiveness of antimicrobial photodynamic therapy (aPDT) against *Candida albicans* (ATCC 90028) using different methylene blue (MB) formulations as photosensitizers. Three MB sources were tested: laboratory-grade methylene blue (MBl) (Sigma-Aldrich, USA), a commercial formulation (MBc) (Evilux™, Fórmula & Ação, Brazil), and a lollipop-based formulation (MBlp) (Doctive™, Brazil). All photosensitizers were adjusted to a final concentration of 150 μM. A pre-irradiation time of 10 min was standardized to ensure adequate uptake of the photosensitizer by fungal cells. Irradiation was then performed using a 660 nm laser (Laser Duo, MMOptics, Brazil) for 6, 9, and 12 min. Each sample was irradiated individually from top to bottom, with the laser tip precisely aligned to the diameter of the well in a 96-well microplate. Standardized fungal suspensions were prepared in Sabouraud Dextrose Broth (Difco Laboratories Inc.) under aerobic conditions at 37°C for 24 h. Fungal suspensions were standardized to $10^7$ CFU/mL after incubation in Sabouraud Dextrose Broth and resuspension in PBS, with cell density adjusted by spectrophotometric transmittance (10–15% at 540 nm). Serial 1:10 dilutions were then performed to reduce the CFU/mL concentration for final quantification. Ten microliters from each dilution were plated onto Sabouraud Dextrose Agar Petri dishes and incubated aerobically at 37°C for 24 h. All samples were plated in triplicate and repeated on three different days (n = 9). The mean values for each group were converted into survival fractions by dividing them by the mean CFU/mL of their respective control group. Data were analyzed using the Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction ($p < 0.05$). The MBl and MBc groups showed a statistically significant reduction starting at 6 min ($p < 0.05$) and did not exhibit further significant reductions with longer irradiation times, nor were there significant differences between the two groups when comparing the same irradiation times ($p > 0.05$). In contrast, MBlp did not demonstrate a statistically significant reduction at any irradiation time ($p > 0.05$). In conclusion, the results demonstrated that aPDT efficacy varied depending on the methylene blue formulation and the light dosimetries still need further adaptations. Acknowledgment: Financial support by the National Council for Scientific and Technological Development (CNPq), Brazil (Grant: 420645/2023-3).

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# Spectral and microbiological evaluation of methylene blue formulations in antifungal photodynamic therapy

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**Keywords***: Candida albicans*; Aggregation; Light-based technology; Photochemotherapy.

The increasing prevalence of antifungal resistance underscores the urgent need for alternative therapeutic strategies. Photodynamic therapy (PDT) has emerged as a promising approach, particularly due to its non-resistance-inducing mechanism of action. This study aimed to evaluate the photodynamic effectiveness of two methylene blue (MB) formulations against *Candida albicans* (ATCC 90028) and to correlate their antifungal activity with their respective absorption spectra. Fungal suspensions were standardized to $10^7$ CFU/mL after incubation in Sabouraud Dextrose Broth and resuspension in PBS, with cell density adjusted by spectrophotometric transmittance (10–15% at 540 nm). Two MB formulations were tested: laboratory-grade methylene blue (MBl, Sigma-Aldrich, USA) and a lollipop-based formulation (MBlp, Doctive™, Brazil), both adjusted to a final concentration of 150 µM. After a 10 min pre-irradiation time, samples were exposed to a 660 nm diode laser (Laser Duo, MMOptics, Brazil) for 6- or 12-min. Irradiation was applied individually, with the laser tip aligned to the diameter of each well in a 96-well plate. Serial 1:10 dilutions were plated on Sabouraud agar and incubated aerobically (37 °C, 24 h). All samples were plated in triplicate and repeated on three different days (n = 9). Colony counts were used to calculate CFU/mL, and survival fractions were determined relative to control groups. Data were analyzed using the Kruskal–Wallis followed by Bonferroni tests ($p < 0.05$). Spectral analysis was performed using a Jasco V-730 UV-Vis spectrophotometer (Jasco, Japan), scanning from 400 to 900 nm with quartz cuvettes. Absorption spectra were analyzed using GraphPad Prism (GraphPad Software, USA). The dimer-to-monomer ratio ($R = Abs_{610nm} / Abs_{660nm}$) was calculated to assess molecular aggregation, a parameter known to influence the photodynamic efficiency of MB. Microbiological results revealed that MBl significantly reduced fungal viability starting at 6 min of irradiation ($p< 0.05$), whereas MBlp did not produce statistically significant reductions at any exposure time ($p > 0.05$). These findings were consistent with spectral data: MBl exhibited a sharp, intense absorption peak centered at 660 nm, optimally matching the emission of the laser source. In contrast, MBlp showed a broader, less defined peak with lower absorbance (Figure 1). The spectral differences suggest possible interference from photosensitizer formulation or molecular aggregation, which may impair light absorption and singlet oxygen generation. In conclusion, the antifungal efficacy of aPDT is strongly influenced by the spectral characteristics of the photosensitizer, PDT efficacy varies depending on the methylene blue formulation. The laboratory-grade MB (MBl) formulation demonstrated superior performance and appears more suitable for clinical application in antifungal photodynamic therapy protocols.

Acknowledgment: Financial support by the National Council for Scientific and Technological Development (CNPq), Brazil (Grant: 420645/2023-3).

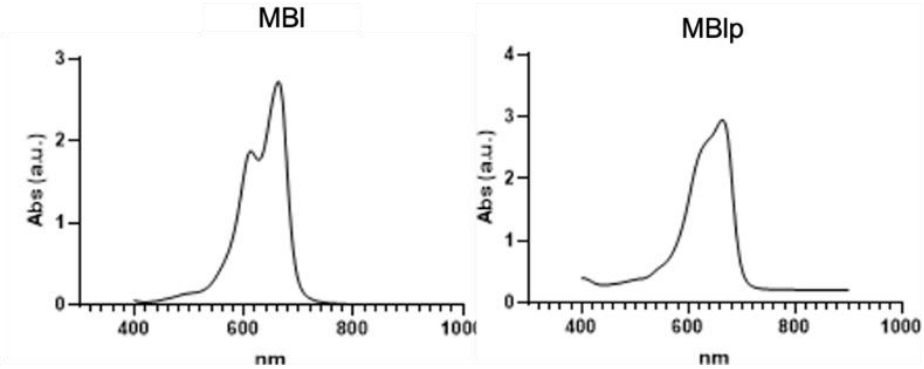


Figure 1– Absorption spectra of methylene blue formulations. laboratory-grade methylene blue (MBl); lollipop-based formulation (MBlp).

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# Correlation between Structure and Properties: Aza-BODIPY Complexes Based on Organoboron Compounds as Singlet Oxygen Photosensitizers

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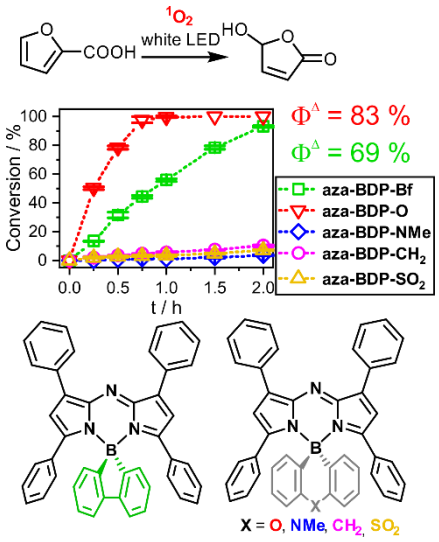
**Keywords:** photosensitizers; singlet oxygen; aza-BODIPY; spiro complexes;

Singlet oxygen ($^1O_2$) is a highly reactive chemical species with strong oxidizing properties, making it valuable in water purification processes, photoinactivation of microorganisms, and anticancer photodynamic therapy. One of the most efficient methods of singlet oxygen generation involves the use of photosensitizing compounds, which, after photoexcitation, undergo intersystem crossing (ISC) from the singlet to the triplet excited state, enabling energy transfer to molecular oxygen. [1]

Our research team specializes in the design and synthesis of boracyclic organoboron compounds, used here as electron donors in heavy-atom-free, spiro aza-BODIPY-based photosensitizers. These systems exploit spin–orbit charge transfer intersystem crossing (SOCT-ISC) as a mechanism to efficiently populate the triplet state.[2, 3] A key feature of this platform is the tunability of the electronic nature of the organoboron core, which allows rational control over intersystem crossing efficiency and singlet oxygen production. Aza-BODIPY incorporating dibenzo[1,4]oxaborinine exhibits a high singlet oxygen quantum yield ($\Phi^{\Delta}$) of 85%, whereas other closely related analogues (dibenzo[1,4]azaborinine) showed negligible activity. This clear structure-activity relationship highlights the critical role of the donor fragment's electronic character in modulating photosensitizing behavior.




Moreover, aza-BODIPY-based complexes outperformed BODIPY analogues in benchmark photochemical oxidation reactions, confirming their superior potential for catalyzing $^1O_2$-mediated processes. Complementary DFT and TD-DFT calculations provided insights into excited-state dynamics and energy transfer mechanisms, elucidating the differences in $^1O_2$ generation efficiencies. Our results provide valuable insights into the molecular design principles for the development of efficient, metal-free photosensitizers for singlet oxygen generation.

Funding for research within the OPUS 20 project "Efficient photosensitizers based on rigid boron-limiting systems as singlet oxygen generators" (2020/39/B/ST4/02370) and Welcome on board project granted by Warsaw University of Technology under the program Excellence Initiative: Research University (ID-UB)..

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**Photodynamic Therapy and Diagnosis**

# Methylene blue-mediated photodynamic inactivation of *Candida auris* biofilms using visible light: A molecular and photobiological study

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**Keywords:** Aloe vera; visible light; super fungus; aPDT.

*Candida auris* (*Candidozyma auris*) causes severe invasive fungal infections (IFIs) with high mortality rates worldwide (40–60%), limiting the treatment options for patients, as it is usually resistant to at least two classes of systemic antifungals (93% are resistant to fluconazole, 35% to amphotericin B, and 7% to echinocandins), causing difficult-to-treat outbreaks in healthcare settings. Effective control and prevention measures against *C. auris* transmission in hospitals are crucial due to its rising incidence and outbreaks worldwide [1]. Antimicrobial photodynamic therapy (aPDT) uses visible light to activate a photosensitizing agent (PS), leading to cell death via photochemical reactions and reactive oxygen species (ROS). Methylene blue (MB), a cationic thiazine dye, is commonly used in aPDT due to its photochemical properties and strong absorption at 630–680 nm, promoting aggregation in target tissues, depending on concentration [2]. In this way, our aim was to analyze the antifungal activity of MB as well as its photodynamic and molecular activity under visible light to combat *C. auris* biofilm. Antifungal activity was analyzed by molecular docking simulations between MB and the apo crystal structure of *C. auris* dihydrofolate reductase (DHFR). aPDT for biofilm produced by *C. auris* CDC B11903 strain involved a $1 \times 10^7$ CFU $mL^{-1}$ suspension incubated with 15–500 mg $mL^{-1}$ of MB solution for 30 min in the dark, followed by irradiation with 400–780 nm light (25 mW $cm^{-2}$, 96 J $cm^{-2}$) for 15, 30, and 60 min at 5 cm of distance from the light source. *C. auris* biofilm inhibition was assessed using a 24-hour culture in 96-well plates, then quantified by crystal violet staining [3], also by qPCR expression of ALS5 and pACT1 genes. Statistical analysis employed two-way ANOVA with Tukey's post hoc test. MB demonstrated a binding energy with *C. auris* DHFR protein of −7.3 kcal/mol, an LE of 0.365, a BEI of 0.026, a Ki value of 4.424 μM, and a fit quality (FQ) value of 0.655. For aPDT, an average biofilm production inhibition of ~56.6% (52.8–59.6%) was observed using 15 mg $mL^{-1}$ of MB, with no difference noted across exposure times. For the 500 mg $mL^{-1}$ MB concentration, a decrease in biofilm production of ~80% was observed, specifically at the 15-minute exposure time. Regarding the ALS5 gene expression profile, a 10x reduction in *C. auris* biofilm production was seen when treated with 500 mg $mL^{-1}$ of MB for 15 minutes, with no gene expression detected at longer exposure times, indicating possible degradation of the genetic material. These results highlight the potential of MB-aPDT as an effective approach for controlling *C. auris* biofilms through exposition of visible light, offering a new therapeutic perspective against this resistant pathogen.

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# Antimicrobial Efficacy and Preservation Potential of Photoactivated ZnO Nanoparticles on Strawberries

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**Keywords:** antimicrobial activity; zinc oxide nanoparticles; photocatalysis.

Strawberries are highly nutritious but perishable fruits prone to microbial contamination and spoilage, limiting their shelf life and posing potential health risks. This study aimed to evaluate the antimicrobial efficacy of photoactivated zinc oxide nanoparticles (ZnO NPs) and assess their impact on the shelf life and antioxidant activity of strawberries.

Methods. ZnO NPs were prepared from a 50% colloidal dispersion and characterized using scanning electron microscopy (SEM), dynamic light scattering (DLS), UV-Vis, and fluorescence spectroscopy. Antibacterial activity was tested against *Escherichia coli O157:H7*, *Listeria monocytogenes* $ATC_{L3}C$ 7644, and *Enterococcus faecalis* MSCL 302. Mid-log phase bacterial cultures were incubated with ZnO NPs ($10^{-5}$ – $10^{-3}$ M) and exposed to 405 nm LED light (6.3 J $cm^{-2}$ – 89 J $cm^{-2}$). Cell viability was assessed via colony counts. SEM was used to examine morphological changes in treated bacteria, and DNA damage was evaluated by an endonuclease assay. Decontamination efficiency was assessed on naturally contaminated strawberries treated with photoactivated ZnO NPs ($5\times10^{-3}$ M). Yeast and microfungi counts were determined via DG18 agar plating. Shelf life was evaluated over 7 days using Kaplan-Meier analysis and spoilage scoring. Total antioxidant capacity was measured using the FRAP method.

Results. Characterization of the ZnO NPs by DLS and SEM revealed an average particle size of ~200 nm, with visible signs of agglomeration. Spectral analyses confirmed typical ZnO optical properties, with a prominent absorption peak at 375 nm and fluorescence emission at ~520 nm.

*In vitro* antibacterial assays revealed that ZnO NPs alone did not affect the viability of *E. faecalis*, *L. monocytogenes*, or *E. coli*. However, significant bacterial inactivation occurred upon exposure to visible light (405 nm), with *L. monocytogenes* showing the highest susceptibility. Clear dependence of the antimicrobial properties of ZnO NPs on the illumination dose was observed. Complete bacterial inactivation to an undetectable level was achieved using specific irradiation doses (≥50 J $cm^{-2}$) and incubation times. SEM analysis showed nanoparticle attachment, cell membrane damage, and intracellular content leakage. DNA integrity assays confirmed oxidative damage in light-treated samples.

The application of photoactivated ZnO NPs on strawberries resulted in a 1.4-log reduction in naturally occurring yeasts and fungi, significantly inhibiting their regrowth over six days. Additionally, treated strawberries displayed an extended shelf life of up to two days compared to the control group, with no adverse effects on total antioxidant activity.

These findings underscore the potential of visible-light-activated ZnO NPs as a safe, effective, and non-destructive method for enhancing the microbiological safety and extending the shelf life of strawberries. Further research should focus on optimizing treatment parameters for commercial-scale applications. However, taking precautionary measures to mitigate potential risks to human health or the environment associated with nanoparticles is essential.

# Energy transfer and quenching in VCP under stress conditions

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Keywords: Quenching, Nannochloropsis, Spectroscopy, VCP

As immobile organisms, plants, algae, and cyanobacteria are constantly exposed to harsh environmental conditions such as high light intensity and extreme temperatures. To survive, they rely on photoprotective mechanisms such as quenching, which safely dissipates excess absorbed light energy as heat to prevent photodamage [1]. When photosynthetic systems exceed their light saturation point, the risk of over-excitation increases, leading to photoinhibition and the formation of reactive oxygen species, which can severely impair photosynthesis [2]. In the absence of effective quenching mechanisms, excess excitation energy would cause irreversible damage to the photosynthetic apparatus, ultimately compromising organismal viability. This underscores the critical importance of elucidating the molecular basis of energy dissipation pathways, which are associated with antenna proteins. One such antenna protein is the Violaxanthin–Chlorophyll-a protein (VCP), a chlorophyll-a-only member of the light harvesting complex (LHC) family with three carotenoids: violaxanthin, vaucheriaxanthin, and vaucheriaxanthin-ester. For this study, we focused on VCP from *Nannochloropsis*, a species known for its wide range of biotechnological applications, including lipid production, oral delivery systems in aquaculture, and as a nutrient-rich feed source for farm animals [3].

Earlier spectroscopic work has shown that carotenoid-to-chlorophyll energy transfer in VCP is highly efficient (~90%) [4], suggesting an important role of quenching under stress conditions. We studied how VCP responds to protein stress, by treating it with increasing urea concentrations and exposing it to high temperatures, using time-resolved fluorescence and ultrafast transient absorption spectroscopy. Under stress, we observed a shorter chlorophyll fluorescence lifetime, indicating increased quenching activity. In the carotenoid absorption bands, a shift was observed under stress conditions, suggesting that the carotenoid binding to VCP was also affected. Interestingly, despite these changes, VCP still showed energy transfer from carotenoids to chlorophylls, although with lower efficiency (50%), highlighting that the protein is robust and resilient under stressful conditions. Also, maintaining decent Car-to-Chl energy transfer efficiency is a sign of light-harvesting, while the shorter Chl-a lifetime is a sign of stress-induced quenching. Thus, we can conclude that VCP optimizes the balance between light-harvesting and quenching.

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# Phthalocyanine-based biocompatible PCL nanoparticles: polymerisation, nanoprecipitation and photoproperties

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**Keywords:** Phthalocyanine; Nanoparticles; Polycaprolactone

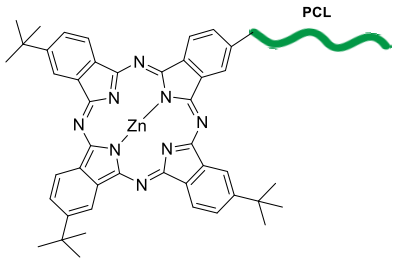
Phthalocyanines (Pcs) have emerged as leading photosensitizers in photodynamic therapy (PDT) owing to extraordinary photophysical and photochemical properties [1]. However, most Pcs often face challenges such as poor water solubility and a propensity to aggregate. To address these issues, a biodegradable and biocompatible polycaprolactone (PCL) star polymer has been previously prepared to encapsulate a NIR absorbing Pc core, the polymer being on the Pc core itself used as the initiators for the ring-opening polymerization (ROP) of ε-caprolactone [2]. This strategy results in the formation of nanoparticles that enhance both solubility and stability, making them more suitable for therapeutic application.

$A_3B$-type phthalocyanines (here ZnPcs) are asymmetrically substituted phthalocyanine derivatives with three isoindole subunits bearing the same substituents (A) and the fourth subunit bearing another type of substituent (B). This asymmetry offers significant advantages for fine-tuning chemical, photophysical, and biological properties, especially for biomedical applications like PDT [3].

To expand the scope of available Pc-based PCL nanostructures, $A_3B$ hydroxylated ZnPcs have been designed to be used as initiators to construct biocompatible Pc-initiated PCL polymers.

Their synthesis and characterization as well as the formation of nanoparticles will be presented.

The Scientific and Technological Research Council of Türkiye (TÜBİTAK) is gratefully acknowledged (project 224Z094).

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# Photo-induced electron transfer in photosensitiser-modified laccase: probing pathways from the surface to the metal centers

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**Keywords:** Laccase; ruthenium complex; porphyrins; photo-induced electron transfer.

Laccase is a robust multicopper oxidase enzyme. Multicopper Oxidases (MCOs) couple the oxidation of a wide variety of substrates to the reduction of dioxygen at two distinct redox centers.[1] Substrate oxidation occurs at a mononuclear type 1 Cu center (T1) located close to the surface,[2] while dioxygen reduction occurs at a buried trinuclear Cu cluster (TNC, composed of a type 2 (T2) and type 3 pair (T3) of $Cu^{2+}$) located 13 Å apart from the T1.[3]
Covalently attaching transition-metal based photosensitiser complexes to the surface of redox enzymes offers then a valuable mean to probe electron transfer (ET) pathways to redox active sites.[4]
This study explores the grafting of a ruthenium-polypyridyl or a zinc porphyrin photosensitizer onto the surface of a multi-copper oxidase to investigate photoinduced electron transfer (PET) processes within the enzyme. Four positions of the photosensitiser relative to the T1 copper and TNC centres of the enzyme are compared (Figure 1).

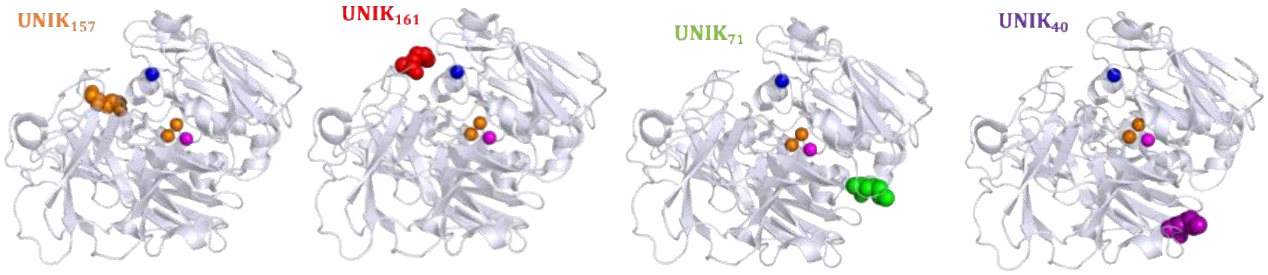


Figure 1. Laccase structural ribbon model of the unique surface-accessible K residues variants (UNIKs) with the K residue coloured according to each variant. T1 Cu ion is a blue sphere, T2 a magenta sphere, and the T3 Cu pair orange spheres.

The positioning of the photosensitiser highlights the roles of distance and intervening medium on PET pathways. Our results reveal photoreduction scenarios where the TNC centre is reduced directly without first passing through the T1 centre. We further illustrate the efficiency and robustness of these hybrid systems through continuous irradiation experiments and laser flash photolysis, shedding light on mechanistic pathways and potential applications in sustainable photocatalysis.

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# Noncovalent Binding of Photochromic Molecules to Lysozyme: Spectroscopic Analysis and Molecular Docking Simulation

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**Keywords:** photochromism; enzyme activity; docking simulation

Photochromic ligands have the potential to reversibly control bioactivities and are valuable tools for the design biomimetic functional materials. In a previous study, we reported that the binding constant of photochromic diarylethene derivatives with hen egg white lysozyme can be reversibly modulated through photoisomerization. In this study, to explore the capability of photochromic ligands for controlling enzyme activity, we synthesized 1,2-bis (2-methyl-3-thienyl) cyclopentene derivatives with carboxyl, methoxycarbonyl, or methylamino groups at the 5 and 5'positions of the thiophene ring as photo-switching diarylethenes. The noncovalent interactions of these diarylethene derivatives with lysozyme were characterized using fluorescence spectroscopy and protein-ligand docking simulations. These diarylethene derivatives exhibited good photochromic properties in 20 mM buffer solutions (pH = 5.0–9.2) containing 3 vol% DMSO in the presence of 1–10 equivalents of lysozyme. The binding constants of the diarylethene derivatives to lysozyme were determined by fluorescence quenching. Enzyme activity was assayed using *Micrococcus luteus* as a substrate, and the kinetic parameters were analyzed according to the Michaelis–Menten model. It was found that the diarylethene with carbonyl groups at the 5 and 5' positions of the thiophene ring (dae**1**) was the most effective in modulating the enzymatic reaction due to its high affinity for lysozyme in the closed form. Analysis of the fluorescence quenching of Trp in lysozyme data revealed that both isomers of dae**1** form a 1:1 complex with lysozyme. The binding constant for the closed form of dae**1** with lysozyme was $10^6$ $M^{-1}$, which is over 10 times larger than that of the open form, regardless of pH. As a result, the catalytic efficiency of lysozyme at the photo-stationary state (313 nm) was reduced to 10% of that observed with the open form. Structural analysis was carried out using MOE molecular dynamics software (CCG Inc.). The molecular docking results and accessible surface area (ASA) calculations revealed that both isomers of dae**1** are located in substrate binding subsite B, and interact with Trp62, which contributes to the bulk fluorescence of lysozyme. Additionally, only the closed form shows an ionic interaction with Arg, leading to the formation of a more stable lysozyme-dae**1** complex.

# Tetrapyrroles In Polymers: Polymerization Monomers vs Grafting onto Polymers

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**Keywords:** Tetrapyrrole; Polymer; Porphyrins; Phthalocyanine; Methacrylate

Tetrapyrroles are organic macrocyclic compounds consisting of four five-membered heterocyclic pyrrole rings. Members of this family, such as porphyrins, chlorins, and phthalocyanines, exhibit unique electronic, photophysical, and coordination properties that enable them to mimic biological functions [1] and support advanced materials applications [2]. Their incorporation into polymeric materials has garnered significant interest for applications including biomedical imaging [3] and photodynamic therapy [4].

This study presents a comparative analysis of two main strategies for integrating tetrapyrroles into polymethacrylate materials: 1) the direct polymerization of methacrylate-functionalized tetrapyrrole monomers, and 2) the post-polymerization of phenol-functionalized tetrapyrroles grafting onto preformed polymer backbones.

The study evaluates the advantages and limitations of each method regarding structural control, loading efficiency, processability, and retention of tetrapyrrole functionality.

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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.

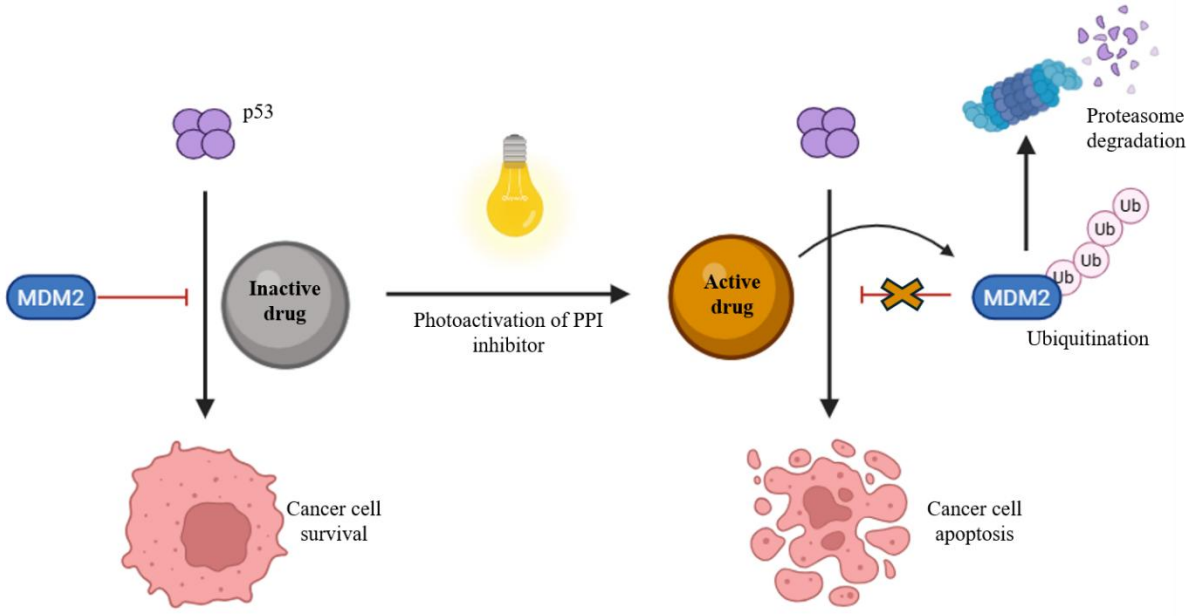


*Fig1. Schematic representation of activation of photoactivatable PPI inhibitor*

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# Did Mr Edison kill Dr Black in the lounge with a light bulb?

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**Keywords:** Skin cancer; attribution; circadian rhythm; prevention

Cancer registration data show that in a century and a half the fraction of the Western populations at risk for skin cancer has gone up by nearly 2 orders of magnitude, even after correction for ageing. Many know for sure that this comes from our excessive engagement in tanning from sun and sunbed, and now we're being punished for it! "Less is better" is the solution. Many others knows for sure that the rise of the skin cancer incidence is simply attributable to overregistration and that therefore the skin cancer trends are not a health problem.

Consequently, the debate on the true cause of the observed trends has stalled and our advice that could help free some scarce health care money and medical or nursing staff remains frozen. Welch has shown that overexposure can explain only a factor of 2 and overregistration a factor 3 [1]. We are overlooking the largest cause! [2]

In this presentation, we categorize 40 possible causes of skin cancer trends and point out the plausible ones. One of them is disruption of the circadian rhythm by the introduction of incandescent light and successors in our domestic evenings [3,4], an issue that is only discussed so far as a niche subject in the separate circadian sessions of this conference. The audience is encouraged to contribute to a discussion on which factors are the most plausible to play a role and help distill a prioritized set of issues that should be addressed by a contemporary skin cancer prevention strategy that is more evidence based and therefore more effective than the current "less is better".

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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 (lower 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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# Chlorella and Cy5: an energy transfer system to improve photosynthetic efficiency

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**Keywords:** Chlorella, cy5, energy transfer

## EXTENDED ABSTRACT

Photosynthetic efficiency depends on the wavelength of the absorbed light, and Photosynthetically Active Radiation (PAR, 400–700 nm) accounts for only about 45% of the total incident solar radiation. Consequently, the maximum theoretical efficiency of the photosynthetic process is around 12%. Among photosynthetic organisms, microalgae show faster growth rates and higher photosynthetic efficiencies compared to terrestrial plants (approximately 3% vs. 0.2–1%). Improving photosynthetic efficiency (PE) is one of the major goals in enhancing $CO_2$ uptake and biomass production. Our research focuses on uncovering the molecular mechanisms behind the interaction between artificial light-harvesting antennas and the photosystem that leads to enhanced PE. Specifically, we aim to understand how the artificial antenna interacts with the photosystem and where this interaction occurs. We used Chlorella sp. as a model organism and Cy5 dye as the artificial antenna. Time-resolved photoluminescence (TRPL) measurements were performed with excitation at 600 nm, where Cy5 absorbs strongly. Two sample types were analyzed: a control sample and algae incubated with Cy5 for 24 hours. A decrease in the fluorescence lifetime of Cy5 in the presence of algae suggested the occurrence of an energy transfer phenomenon. This observation was supported by fitting the TRPL data of the Cy5-algae system using the TRPL profiles of isolated Cy5 and algae. In samples that were not washed (thus containing free dye), this fitting approach reproduced the observed data well. However, after centrifugation (removing unbound Cy5), the same fitting failed. This indicates that the observed lifetime changes are not due to a simple overlap of Cy5 and algal signals, but rather to a genuine energy transfer from Cy5 to the photosynthetic machinery. Additionally, hyperspectral excitation microscopy was performed in the presence and absence of Cy5, collecting emission above 700 nm, where Cy5 does not emit. Upon excitation at Cy5-absorbing wavelengths, cy5 excitation was observed in dye-treated samples, further indicating energy transfer from Cy5 to chlorophyll. We also conducted fluorescence lifetime imaging microscopy (FLIM), exciting at 600 nm (absorbed mainly by Cy5) and at 450 nm (absorbed only by chlorophyll). This allowed localization of the dye in the membrane of the cells and within the chloroplasts, which are also membrane-bound. To confirm the energy transfer, we first saturated chlorophyll excitation using 450 nm light and then excited with 600 nm light. We observed increased emission and longer lifetimes for cy5, consistent with energy transfer from Cy5 to chlorophyll in a system where the acceptor is saturated. In conclusion, we successfully used TRPL, FLIM, and hyperspectral excitation microscopy to demonstrate an energy transfer phenomenon in the Chlorella–Cy5 system.

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# Fluorescence spectral imaging of agar plate cultures

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**Keywords:** agar plate cultures; fluorescence; spectral imaging

Fluorescence spectral imaging provides information about location, size, and the distribution of fluorescent pigments in bacterial colonies. This information could help in the differentiation of species and colony evolution monitoring.

Four different microbial species were included in this study: *S. aureus* ATCC 25923, *P. aeruginosa* PAO1, *E. coli* ATCC 25922, *and C. albicans* ATCC 10231. They were inoculated on tryptic soy agar plates overnight and imaged on days 1, 2, and 3 by a custom laboratory hyperspectral system. The illumination source was a 395 nm 1130 mW laser diode (M395L5, Thorlabs, USA), and the hyperspectral imaging system was built around an imaging spectrograph (ImSpector V10E, Specim, Finland) with a 400–1000 nm spectral range and approx. 3 nm spectral resolution. Fluorescence images were normalized to the incident illumination source intensity to ease comparison of the spectra. Spectra were smoothed using median filtering with a window size of 10 pixels (Matlab 2023b, MathWorks, USA).

All fluorescence images show fluorescence in the spectral range 430 – 550 nm with a peak at 485 nm in medium and colonies, which represents the background fluorescence of the plates. *S. aureus* fluorescence also features a relatively narrow fluorescence peak at 777 nm (FWHM is approx. 13 nm), which is most prominent in the center of the colony and diminishes towards the colony boundary (Fig. 1b). This peak most likely corresponds to bacteriochlorophyll fluorescence [1]. The image of the corresponding spectral band 770–780 nm (Fig. 1c) clearly shows the location of the colonies, while the areas of the medium are black. The peak prominence is increasing with the colony age. *P. aeruginosa*, on the other hand, features a broadband fluorescence in approx. 430–600 nm region with the peak at approx. 470 nm. The fluorescence expression significantly increases with colony aging. This fluorescence is most likely due to pyocyanin fluorescence [2], which fluoresces in the 400 – 600 nm spectral range [3]. The *E. coli* does not present any fluorescence, while *C. albicans* show a weak fluorescence peak at 775 nm, which increases gradually with colony age.

In our study, we demonstrated that fluorescence spectral imaging can be used for imaging of bacterial colonies, which resulted in the detection of fluorescent bacteria and differentiation based on the location and shape of the fluorescence emission peaks.

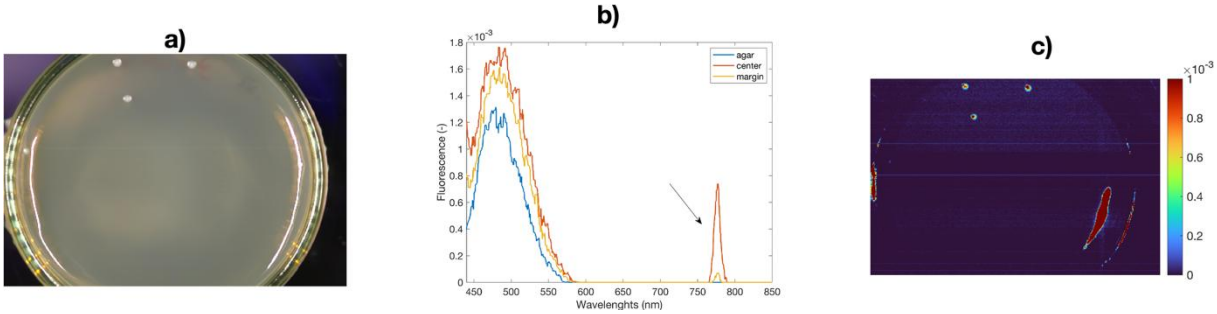


**Figure 3:** *S. aureus* cultures on day 1 after inoculation. (a) RGB images reconstructed from broadband illumination. (b) Fluorescence spectra of agar, in the center and at the boundary of the colony. (c) Image at 770 nm – 780 nm spectral band.

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# Vanillin antioxidant behavior under UV irradiation: a paradox

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**Keywords:** vanillin, UV-A radiation, cytotoxicity, ROS

Reactive oxygen species (ROS) are intermediate species formed during both the normal metabolic process and under stress situations. Excessive accumulation of ROS, in particular under exposure to artificial or natural electromagnetic radiation, exceeds the normal capacity of cells to neutralize these harmful species and, therefore, cause damage to biomolecules and affects cell metabolism.[1] Research has shown that the use of antioxidants is effective in reducing the damage caused by ROS. However, the antioxidant capacity of a given compound is typically assessed in the dark and often does not consider exposure to solar or artificial radiation.

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is well-known as a flavoring agent and also for its antioxidant properties.[2] However, it is unknown whether it retains its antioxidant capacity when exposed to electromagnetic radiation. In this study, the cytotoxicity and phototoxicity of vanillin were evaluated in HeLa cells, that were incubated with vanillin (0 - 4.3 mM) and exposed to UVA radiation ($\lambda$ = 365 nm).

The formation of ROS was assessed using a fluorescent probe based on 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). Results revealed that in the cells exposed to UV-A radiation in the presence of vanillin the generation of ROS was much higher than in the cells kept in the dark.

The cytotoxic effect on Hela cells, evaluated by the Tukey test, was observed at a vanillin concentration of 0.8 mM, the same value being for cells exposed to UV-A radiation or kept in the dark.

These findings suggest that although vanillin amplifies ROS production under UVA radiation, it does not exacerbate cell death, indicating that ROS accumulation does not increase cytotoxicity under these experimental conditions.

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# Harnessing the potential of a NIR emitting BODIPY Dye in bacterial Bio-Hybrid photoenzymes for solar energy conversion applications

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

Photosynthesis involves the conversion of solar radiation to metabolic energy through a multi-protein array shared by plants, algae, cyanobacteria and photosynthetic bacteria. The photochemical core of the machinery known as the Reaction Centre (RC) is responsible for conversion of photons collected by light harvesting antenna complexes into charge separated states, thus allowing redox reactions and photosynthesis to occur.

However, light harvesting antenna complexes are labile when the reaction center photoenzyme is isolated from the photosynthetic bacterium for possible biophotovoltaic applications. Hence, the approach of covalent binding with tailored organic dyes capable of harvesting sunlight and transferring energy to isolated bacterial RC photoconvertes without perturbing their biological activity has been demonstrated to be a suitable promising strategy to develop biohybrid materials for solar energy conversion applications. [2-4]

On this ground, NIR emitting BODIPY based dyes [5] have never been explored so far as organic light harvesting molecules for bacterial RC bioconjugation. Here we report the synthesis of new BODIPY antenna molecules, their photophysical characterization and its possible bioconjugation to the RC of *Rhodobacter Sphaeroides* bacterium, disclosing also the possibility to modulate the antenna light harvesting capability depending on pH.

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# Multiscale Simulation of the Fucoxanthin Chlorophyll a/c Protein Complex from the Diatom *C. Gracilis*

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**Keywords:** Diatoms, Molecular Dynamics Simulations, Excited States

Diatoms are a diverse group of algae with an important biological role, producing at least 20% of the oxygen in our atmosphere through photosynthesis. Recently, the crystal structure of *Chaetoceros gracilis* was resolved[1], making it a valuable model system to investigate light harvesting and energy transfer processes. Particular attention has been given to the fucoxanthin chlorophyll protein (FCP) antenna complexes, which contain pigments such as chlorophyll a (Chl a) and chlorophyll c (Chl c). Building on previous studies[2], a multi-scale computational approach has been employed to bridge the classical and quantum perspectives by combining molecular mechanics (MM) simulations with hybrid quantum mechanics/molecular mechanics (QM/MM) methods using the computationally efficient Density Functional Tight Binding (DFTB) approach for the ground state dynamics and excited state energy calculations. This framework enables the exploration of the conformational dynamics of the system and the molecular interactions involved in photosynthesis, while also assessing the influence of environmental and structural changes. Furthermore, the effect of pH variation is being investigated, as earlier research[3] has shown that a decreased pH value can significantly impact the role of Chl c, resulting in shifts in the energy level landscape within the complex.

Based on these simulations, excited state properties have been analyzed using multi-scale computational methods to predict the excitation energies, excitonic couplings, and spectroscopic features such as spectral densities and absorption spectra, which are compared to experimental results for validation. Additionally, the electric field effect induced on the chlorophyll c pigments by the protein environment is being investigated, following previous work[3] that emphasizes its importance in the energy transfer process.

In conclusion, this work aims at deepening our understanding of the quantum mechanical principles that govern photosynthesis and the light harvesting process. Ultimately, the insights gained from this research may contribute to the development of more efficient and sustainable energy technologies.

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# Surface-Engineered Gold Nanoparticles for Improved Electron Transfer in Photosynthetic Biohybrids

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**Keywords:** Biophotovoltaics (BPVs); Extracellular electron transfer (EET); Biohybrid; Nanoparticles;

Shedding light on the interaction between inorganic nanoparticles (NPs) and living microorganisms is at the basis of the development of biohybrid technologies with improved performance.The use of intact microbial cells as biocatalysts for the development of bioelectrochemical systems has found several applications in recent years,[1] going from localized power generation[2] to (self-powered) biosensing[3, 4] and bioelectrosynthesis of high-value products.[5] In this context, biophotovoltaics (BPVs) represent attractive technologies for cost-effective and sustainable electricity generation.[1, 2] Despite their potential, BPVs face challenges in industrial application due to issues with stability and efficiency. Nanomaterials offer a pathway to enhance these systems, leveraging their unique properties to improve extracellular electron transfer (EET) and light harvesting. Metal NPs can act as both light absorbers and conductive bridges, improving extracellular electron transfer (EET) and charge extraction. However, the mechanisms of NP–bacteria interaction remain underexplored. Herein, we investigate the interaction of water dispersible and rationally designed Au NPs, designed with specific surface properties, with metabolically active photosynthetic purple bacterial cells (Rhodobacter capsulatus) monitoring the effect of surface functionalization on the EET process at the biotic-abiotic interface. The NPs are designed to be small enough and have tailored surface properties posely to interact suitably with bacteria[5] while not resulting cytotoxic thereby improving photoelectrochemical conversion and transport efficiency at the bacteria/NP/electrode interface.

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# Photophysical evaluation of a porphyrin-based photosensitizer for the generation of ROS induced by light: A physicochemical model towards photobiological applications

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**Keywords:** Photobiology, Photosensitizer, Porphyrin, Fluorescence spectroscopy

This research explores the photophysical characteristics of a novel porphyrin-based photosensitizer (PS-47), highlighting its capacity to produce reactive oxygen species (ROS) upon light activation in aquatic environments. This study functions as a physicochemical model system to facilitate early-stage screening and comprehension of light-matter interactions pertinent to photobiological and therapeutic applications.

PS-47 was analysed by UV–Vis absorption spectroscopy to ascertain its molar extinction coefficient and peak optical response. Steady-state and time-resolved fluorescence spectroscopy were employed to assess emission characteristics, quantum yield, and excited-state durations under 660 nm laser excitation. Reactive oxygen species (ROS) generation was evaluated in aquatic environments utilizing the ROS-sensitive fluorescent probe DCFH-DA, with emission intensity measured spectroscopically in real time.

The photosensitizer demonstrated significant absorption at 660 nm, exceptional photostability, and a fluorescence emission peak approximately at 690 nm. Laser irradiation at 100 mW/cm² resulted in a time-dependent enhancement in DCF fluorescence, signifying effective ROS production. The results indicate the appropriateness of PS-47 as a candidate for light-induced oxidative regulation.

In conclusion, our physics-based methodology offers a reliable and manageable framework for assessing novel photosensitizers in physiologically pertinent optical environments. The results advance the creation of next-generation agents for photodynamic studies, nanophotonics, and prospective photobiological applications.

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# NIR-Activated Gold Nanorods for Light-Induced Thermal and Oxidative Modulation: Advancing Photobiological and Nanotechnological Applications

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**Keywords:** Photobiology, Gold nanorods, NIR light, Nanotechnology

This work conducts a photophysical and photothermal analysis of near-infrared (NIR)-responsive gold nanorods (GNRs) to assess their applicability in nanoscale photobiological systems. The study examines their efficacy in light-driven heat conversion and photoinduced oxidative reactions, which are essential mechanisms in developing biomedical and photodynamic nanotechnologies.

Gold nanorods with an average aspect ratio of around 3.5 were synthesised using a seed-mediated growth technique and enclosed in a silica shell to improve colloidal stability. Their optical response was analysed by UV–Vis–NIR spectrophotometry, indicating a pronounced plasmonic peak at around 808 nm. For thermal analysis, suspensions of GNRs were subjected to irradiation with a continuous-wave 808 nm laser (1 W/cm²), and the resultant temperature increase was documented using thermal imaging and integrated micro-thermocouples. The formation of reactive oxygen species (ROS) in aqueous media, pertinent to photobiological signalling and photodynamic treatment, was assessed utilising the DCFH-DA fluorescent probe.

The GNRs demonstrated a wide absorption spectrum (FWHM ~90 nm) and a photothermal conversion efficiency of 40%. Irradiation resulted in a temperature rise of around 16°C within 5 minutes and a significant increase in fluorescence intensity, signifying the generation of reactive oxygen species under optical stimulation. These outcomes illustrate the potential of GNRs to function as photothermally active and reactive oxygen species-generating platforms in biologically friendly environments.
In conclusion, our physics-based and biologically pertinent method substantiates that gold nanorods triggered by near-infrared light possess significant potential as multifunctional agents for photobiological regulation, nanotherapeutic advancement, and optical modulation of cellular microenvironments.

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# Photophysical Properties and In Vitro Phototoxic Effect of Trifarotene

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**Keywords:** Phototoxicity; Retinoids; Photophysics; UV radiation

Trifarotene is a fourth-generation topical retinoid approved by the U.S. Food and Drug Administration (FDA) for the treatment of lamellar ichthyosis and acne vulgaris. [1] However, its concomitant use with sun exposure may promote the onset of adverse effects. These manifestations have been attributed to various mechanisms, including skin irritation—which can compromise the skin's natural photoprotective capacity and enhance the harmful potential of ultraviolet (UV) radiation—and the reduction of stratum corneum thickness, which weakens the natural barrier against UV radiation. [2]

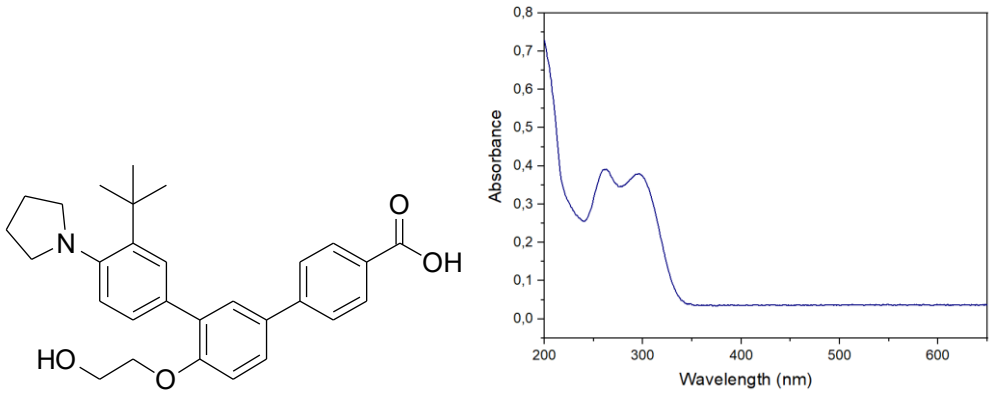


***Figure.*** *Left, chemical structure of trifarotene molecule. Right, UV-vis spectra of trifarotene in acetonitrile.*

Furthermore, as shown in Figure, trifarotene exhibits significant absorption in the UVB/UVA range. Therefore, like other topical drugs, it may act as a photosensitizer, facilitating photochemical interactions with cutaneous biological components and triggering phototoxic or photoallergic reactions. The aim of the present study is to assess the phototoxic potential of trifarotene. In the first stage, we focused on characterizing its photophysical properties to gain deeper insight into its excited states and its ability to induce biomolecular damage. To this end, we conducted experiments involving fluorescence (both steady-state and time-resolved emission), phosphorescence, and transient absorption spectroscopy across timescales ranging from nanoseconds to microseconds.

In the second stage, we evaluated its in vitro phototoxicity using the assay to detect cytotoxic effects induced by ultraviolet radiation.

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# Monitoring light-dependent biomolecular interactions with a novel front-illuminated surface plasmon resonance (fiSPR) biosensor

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**Keywords:** Light-responsive proteins; Kinetic analysis; Protein-protein interactions; Protein-DNA interactions;

Important physiological processes are based on biomolecular interactions mediated by photosensory proteins and light, including vision for animals, growth and development for plants, and adaptation to changing environmental conditions for bacteria. For instance, gene expression in the bacterium *Erythrobacter litoralis* is regulated by EL222, a blue-light photoreceptive transcription factor with applications in optogenetics [1]. Engineered light-responsive proteins, such as interleukin-receptor pairs containing photocaged non-canonical amino acids [2], have also emerged as prospective tools in biotechnology and biomedicine for on demand photoregulation of cell signaling pathways. Despite extensive studies on natural and synthetic light-responsive proteins, the thermodynamic and kinetic interrogation of their binding processes remains challenging, due to the need for simultaneous monitoring and *in-situ* illumination of the interacting partners. Here, we develop a novel front-illuminated surface plasmon resonance (fiSPR) biosensor for real-time and label-free monitoring of interactions involving photofunctional proteins. The fiSPR biosensor combines a backside light source, which enables surface plasmon excitation, with a frontside light source and an advanced microfluidic system, which permits controlled *in-situ* activation and delivery of biomolecules. We implement the fiSPR biosensor to interrogate light-driven protein-protein and protein-DNA interactions involving EL222 [3, 4], including the determination of both association and dissociation rate constants for EL222 dimerization and DNA binding. Furthermore, we propose a novel mechanism to describe EL222-DNA complex formation, in which EL222 self-assembly occurs prior to DNA recognition. These results demonstrate the potential of the fiSPR biosensor as a tool for investigating the affinity, specificity, and kinetics of biomolecular binding equilibria affected by light.

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# Impact of Photosensitized Modifications of Monoclonal Antibodies on their Biological Activity and Safety

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**Keywords:** Monoclonal antibodies, photostability, photosensitized oxidation

## EXTENDED ABSTRACT

Monoclonal antibody (mAb)-based therapies are highly valued for their target specificity, low toxicity, and capacity to elicit durable antitumor immune responses. However, their therapeutic efficacy depends critically on maintaining structural stability throughout production, storage, and administration. Among environmental stressors, light exposure poses a significant risk due to the photoreactivity of certain amino acid residues and formulation components. Photoinduced degradation can lead to oxidative modifications, aggregation, fragmentation, and increased immunogenicity [1,2].

In this study, we systematically investigated the impact of controlled photosensitization on two therapeutic mAbs, Atezolizumab and Durvalumab, using ruthenium(II) tris-bipyridyl ($Ru(bpy)_3^{2+}$) as a model photosensitizer [3]. Under aerobic conditions, photoactivation of $Ru(bpy)_3^{2+}$ induced singlet oxygen ($^1O_2$)-mediated oxidation. In contrast, in the presence of persulfate ($S_2O_8^{2-}$), a photogenerated radical mechanism was favored, resulting in pronounced mAb aggregation.

The resulting physicochemical and functional changes were characterized using spectroscopic methods, SDS-PAGE, mass spectrometry, and receptor blockade bioassays. Our findings delineate distinct oxidative pathways and their differential impact on antibody stability and function. This mechanistic insight can guide the development of more robust mAb formulations, minimizing light-induced degradation and preserving therapeutic activity.

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# INTERACTION OF PHOTOSENSITIZER Mg-CHLOROPHYLLIN AND METAL-BASED QUANTUM DOTS

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**Keywords:** nanoparticles; aqueous media; autofluorescence; spectroscopy.

Magnesium chlorophyllin (Mg-Chl), a photoactive tetrapyrrolic derivative of chlorophyll, is extensively utilized as the natural food colorant E140. This compound exhibits the ability to act as a chlorin-type photosensitizer in the photodynamic therapy, as well as an antioxidant [1]. Consequently, it finds applications in diverse research areas within both the medical and food industries. The tendency of Mg-Chl to either generate reactive oxygen species when exposed to light or to inactivate them depends on environmental characteristics. However, despite their widespread use, the efficacy of both Mg-Chl and many photosensitizers is limited by their **photophysical properties** and the ability to retain these properties in various biological environments. Like many photosensitizers, Mg-Chl is prone to degradation when exposed to light. Thus, increasing attention is being paid to **quantum dots (QDs)** – nanoparticles [2] capable of effectively interacting with bioorganic molecules and modifying their properties, opening up new application possibilities. Although metal-based QDs and chlorophyll derivatives have been studied separately, their interactions at the nanoscale are not fully understood. When QDs absorb light, they can transfer that energy to nearby molecules. This may either enhance the fluorescence of these molecules or induce the formation of reactive oxygen species (ROS), which may cause their photodegradation.

**The aim of this study** is to investigate the effect of interaction between **metal-based quantum dots** and **Mg-chlorophyllin** on their **photostability** and photomodification in an aqueous medium and in the presence of **bovine serum albumin (BSA).** Spectroscopic methods were used to evaluate how this interaction alters the fluorescence, absorption, and other optical properties of metal-based QDs and Mg-Chl and how they influence each other at a molecular level. Additionally, we have showed how both Mg-Chl and QDs (individually and in combination) maintain their optical properties under light irradiation. Studies with BSA were performed to reveal how the Chl-QDs complex behaves in a protein-rich environment. This is critically important for applying Chl-QDs complexes in biomedicine (e.g., for imaging, therapy) or for understanding their behaviour in living organisms. Ultimately, enhanced photostability and optimized optical properties would enable the development of more sensitive and reliable biosensors for diagnostics or environmental monitoring.

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# REDOX PROTEOMICS OF KERATINOCYTES EXPOSED TO UVA LIGHT AND ANALYSIS OF OXIDATIVE MODIFICATIONS

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**Resume.** Singlet oxygen ($^1O_2$), the excited state of molecular oxygen, is a well-known oxidant of double bonds in aromatic rings and free thiol groups. Thus, in biological systems, proteins containing tryptophan, tyrosine, methionine, histidine, and cysteine residues potential targets of this reactive species[1]. This study aims to investigate the redox proteome of immortalized human keratinocyte cells (HaCaT) exposed to UVA light, focusing on oxidative protein modifications and the metabolic pathways affected[2]. HaCaT cells were cultured to approximately 80–90% confluence and plated in 35 mm dishes, each containing approximately $1.0 \times 10^6$ cells. Samples were irradiated using a solar simulator equipped with a UVA filter for 30 minutes (6 J/cm²), while controls were maintained in the same conditions without light exposure. Immediately after irradiation, the cells were lysed in 100 mM ammonium bicarbonate buffer containing protease inhibitors, 0.5% sodium deoxycholate, and 50 mM iodoacetamide to alkylate all initially free thiols. Subsequently, the samples were treated with 8 M urea and incubated with 10 mM dithiothreitol to aid in protein denaturation and reduction of remaining disulfide bonds. Then, 80 mM N-ethylmaleimide was added to the solution at pH 6 to block the newly exposed thiols derived from disulfide cleavage or reversible oxidations. Proteins were then digested with trypsin (1:25, enzyme:protein ratio) overnight at 37°C. All washing steps were performed using the FASP (*Filter-Assisted Sample Preparation*) protocol before peptide injection into a nano-flow liquid chromatography system coupled to an Orbitrap Lumos mass spectrometer (nLC-MS/MS) using data-dependent acquisition (DDA). Following analysis with MaxQuant, Perseus, and RStudio, peptides with oxidative modifications were identified and compared to those from the control group. The comparison between initially alkylated free thiols and those reduced by DTT enabled relative quantification of oxidations induced by $^1O_2$ during UVA irradiation. In addition, enrichment analyses of redox-relevant proteins involved in the oxidation process were performed to identify metabolic pathways affected during UVA exposure and to establish correlations regarding the role of singlet oxygen in redox stress, and in affecting essential cellular signaling functions.

**Keywords.** Redox Proteomics, UVA Exposure, Singlet Molecular Oxygen, Mass Spectrometry.

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# Molecular insights into the DNA binding/unbinding of DHICA melanin monomer and its dioxetane

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**Keywords:** Chemiexcitation; dioxetanes; DNA binding/unbinding; enhanced sampling molecular dynamics

## EXTENDED ABSTRACT

Under oxidative stress, melanin degradation leads to the formation of reactive intermediates such as 5,6-dihydroxyindole-2-carboxylic acid (DHICA), which, upon interaction with peroxynitrite, can give rise to a dioxetane species. These dioxetanes decompose into carbonyl compounds in triplet excited states, which can transfer the excess of energy to DNA nucleobases and lead to the formation of cyclobutane pyrimidine dimers (CPDs) [1]. This phenomenon represents a non-light-mediated pathway to DNA damage and has been linked to both melanin derivatives and other biologically relevant indole-containing molecules such as tryptophan, melatonin, and serotonin [2].

In this work, we studied the interaction of DHICA and DHICA-derived dioxetane with DNA. Using enhanced sampling techniques, such as OPES flooding methods [3], the unbinding kinetics were characterized. The simulations revealed residence times on the order of microseconds and multiple unbinding pathways, highlighting the potential for efficient triplet energy transfer when intercalated within DNA base pairs.

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# Near-Infrared Direct Detection of Singlet Oxygen Generation From UVA-Excited 6-Thioguanine

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**Keywords**: 6-Thioguanine; Singlet Molecular Oxygen; DNA; Photosensitization.

**Introduction**: 6-Thioguanine (6-TGua) is a thiopurine widely used as a cytostatic drug in the treatment of autoimmune diseases and transplant patients. When internalized by cells, 6-TGua is metabolized via the purine salvage pathway and incorporated into DNA. Clinical studies have shown that patients receiving thiopurine therapy have an increased risk of developing skin cancer [1]. In contrast to guanine (Gua) or other canonical bases, 6-TGua displays a maximum absorbance at 342 nm, making it susceptible to photoexcitation by UVA radiation, which is abundant in sunlight. Although the exact mechanisms leading to skin cancer in these patients remains unclear, one hypothesis is that DNA-incorporated 6-TGua acts as a photosensitizer, generating singlet molecular oxygen ($^1O_2$) within DNA [2]. **Objective**: This study aimed to unequivocally demonstrate the generation and quenching of $^1O_2$ from and by 6-TGua, through the direct spectroscopic detection of the monomolecular light emission characteristic of $^1O_2$ at 1270 nm. **Materials and Methods**: A steady-state approach was based on $^1O_2$ chemiluminescence to determine the quantum yield of its production ($\Phi_{^1O_2}$). Quenching experiments were performed using the N,N-di(2,3-dihydroxylpropil)-1,4-naphthalenedipropanamide-1,4-endoperoxide ($DHPNO_2$) as a clean source for the generation of $^1O_2$ in a steady-state concentration. The total $^1O_2$ quenching rate constant ($k_t$) was determined by the Stern-Volmer relationship by using various concentrations of the following compounds: the nucleobase 6-TGua, its related 2'-deoxyribonucleoside (6-TdGuo), the canonical 2'-deoxyguanosine (dGuo) and its oxidation product 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo). The results obtained for the thio-compounds were compared with the ones for dGuo and 8-oxodGuo, that are exclusive targets in DNA for $^1O_2$ oxidation and already have documented values of $k_t$ [3]. **Results and Discussion**: The $\Phi_{^1O_2}$ values obtained for 6-TGua (0.18) and 6-TdGuo (0.11) were consistent with previously reported data [4]. The $k_t$ values ($1.5 \times 10^7$ L × $mol^{-1}$ × $s^{-1}$ for 6-TGua and $1.1 \times 10^7$ L × $mol^{-1}$ × $s^{-1}$ for 6-TdGuo) indicated that both compounds exhibit quenching capabilities comparable to those of dGuo ($6.3 \times 10^6$ L × $mol^{-1}$ × $s^{-1}$) and 8-oxodGuo ($5.4 \times 10^7$ L × $mol^{-1}$ × $s^{-1}$). These findings contribute to a better understanding of the potential reactivity of DNA containing 6-TGua towards $^1O_2$ in a biological context. **Conclusion**: Our results provide direct evidence of $^1O_2$ generation and quenching by 6-TGua and 6-TdGuo upon UVA irradiation, supporting the hypothesis that photosensitization by DNA-incorporated 6-TGua may contribute to the molecular mechanisms underlying skin cancer development in thiopurine-treated patients.

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# How does sunlight influence zebrafish development?

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## ABSTRACT

Exposure to sunlight is a fundamental component of life on Earth and has influenced the evolution of nearly all terrestrial and many aquatic species. The ability to sense light is essential for timing the internal biological clock and directly affects growth, survival, and behavior. Furthermore, organisms have developed adaptive mechanisms to balance the physiological benefits of sunlight exposure, such as Vitamin D synthesis, against the risks of UV-induced DNA damage.

Over the last decade, photosensitive cells have increasingly been identified in a variety of tissues beyond the visual system, including the skin, heart, lungs, liver, and adipose tissue. In adult zebrafish, cells dissected from skin, fins, and multiple internal organs showed light entrainment capabilities when cultured independently *in vitro* [1]. This suggests that all cells in the body may have some ability to detect and respond to light stimuli. However, many of the responsible photoreceptors and their downstream effectors are unknown. In addition, studies using zebrafish larvae have shown that exposure to different wavelengths within the visible light spectrum greatly impacts viability. Larvae raised exclusively in blue or violet light displayed normal growth and survival, whereas larvae raised exclusively in red light were stunted and did not survive more than 20 days post hatching [2]. Visible light represents approximately 45% of total solar radiation as compared to less than 5% UV, yet the physiological importance of visible light wavelengths is far less understood.

Because we still do not fully understand which cells are responsive to light, our goal is to define all light-responsive cells in the zebrafish. We are using a recently described whole-animal single-cell RNA-seq approach called sci-Plex. Zebrafish larvae are treated with acute exposure to visible light at two stages of development, 24 hours and 5 days post fertilization, followed by whole-larva dissociation and sci-Plex profiling. Preliminary data has confirmed the feasibility of this approach, and we are now optimizing and scaling up the method. The data obtained from these profiles will allow us to map which cells are responding to light within the larval zebrafish and pinpoint the underlying gene expression changes, potentially leading to the identification of novel photoreceptor pathways. Understanding the mechanisms of light-sensing and the critical wavelengths involved in these pathways will provide a basis for further study of photobiology in human health and disease.

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# Structures and properties of phthalocyanines for PDT

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**Keywords:** Phthalocyanine; Photodynamic; Photosensitiser; Structure; Properties

The photoproperties of phthalocyanines is strongly related to their structure and can be, if not controlled, at least tailored. Fine structural modifications of a phthalocyanine's structure can have huge implications in terms of photoproperties. External factors can be implemented to further modulate these properties. Beyond the most obvious substitution pattern, other ways to customize their photoproperties will be critically presented.

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# Hypericin and $Na_2EDTA$ against tomato infections: using a checkerboard assay to tailor photosensitizer formulations

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**Keywords:** Photodynamic Inactivation, checkerboard assay, hypericin, white light, plant protection

The pathogens *Clavibacter michiganensis* (bacterial canker) and *Pseudomonas syringae* (bacterial speck) pose significant threats to tomato production. They can enter tomato fruits through lesions or contaminate the plant while flowering, which infects the seeds and leads to vertical transmission of the disease. Conventional infection management relies on copper-based bactericides and antibiotics, but these face challenges such as antimicrobial resistance and regulatory restriction [1,2]. In this pilot study we explored tailoring of suitable photosensitizer formulations for Photodynamic Inactivation of Gram-positive *C. michiganensis* and Gram-negative *P. syringae*. Therefore, we tested combinations of the natural photosensitizer hypericin in water soluble form (environmentally friendly derivative, applied for a patent) and the cell wall permeabilizing agent $Na_2EDTA$ in a checkerboard assay layout. Samples were illuminated using white light from a commercial plant growing lamp (130 W $m^{-2}$, 100 J $cm^{-2}$). Fractional inhibitory concentration (FIC) indices were calculated for all samples, that on average showed less than 10 colonies in 3 replications. Hypericin can photoinactivate *C. michiganensis* with a concentration of 3.125 µM without $Na_2EDTA$. Yet, a synergistic effect (FIC index 0.375) was determined when phototreating the Gram-positive pathogen with 0.781 µM hypericin together with 625 µM $Na_2EDTA$. Photoinactiavtion of the Gram-negative *P. syringae* requires the addition of $Na_2EDTA$ for hypericin being photoactive. Once more, a synergistic effect (FIC = 0.031) was achieved with relatively low concentrations of 0.391 µM hypericin plus 78.125 µM $Na_2EDTA$. These outcomes suggest that water-soluble hypericin formulated with $Na_2EDTA$ might represent an alternative to antibiotics in tomato horticulture. Further, the findings highlight the potential of checkerboard assays for tailoring photosensitizer formulations for a specific application, allowing for testing of synergistic combinations of photosensitizer and additives in a high throughput manner. Upcoming work will assess tomato plant safety, focusing on oxidative stress and include other options for $Na_2EDTA$ as cell wall permeabilizing agent.

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# Bacterial photosynthesis in non-aqueous solvents

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**Keywords:** bacterial photosynthesis, Deep Eutectic Solvents, membrane protein, photosynthetic system

Deep eutectic solvents (DESs) are two-component solvents characterized by a eutectic composition having a melting points temperature consistently lower than those of individual components [1]. The formation of a eutectic solution descends from the strong interaction between the components, leading to a low ordered structure that maintains them liquid at room temperature and sustains chemical stability of the molecules involved. DESs are formed by a hydrogen bond acceptor molecule and one hydrogen bond donor or Lewis acids and bases. Most DESs are not toxic, generally low-cost, and their preparation is easy and straightforward. Among the numerous DESs described in literature [2], choline chloride (ChCl) and urea (U) are very common constituents.

DESs have been shown to maintain the catalytic activity of the membrane photosynthetic enzyme – the reaction center (RC) – obtained from the purple non sulphur bacterium *Rhodobacter (R.) sphaeroides* [3] and common water-soluble proteins such as lysozyme [4].

In this study, we extended previous studies for evaluating the biocompatibility of DESs toward chromatophores, photosynthetic membrane vesicles containing whole photosynthetic apparatus, *i.e.* RC and light-harvesting complexes. Chromatophores are isolated from the mutant strain R26 of *R. sphaeroides*. Chromatophores integrity and stability was investigated in ChCl:U (1:2) by UV-Vis-NIR spectroscopy.

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# Effect of UV radiation on dermal stem cell differentiation to melanocytes

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**Keywords:** melanoma; dermal stem cells; UV; differentiation

**Introduction:** Malignant melanoma is the most aggressive and lethal form of skin cancer, with its main etiological factor being exposure to ultraviolet (UV) radiation. While UV-radiation is well-established as a significant cause, the exact cell of origin for melanoma remains elusive. There are two prevailing theories regarding the cells of origin of melanoma: the classical theory, which suggests that melanoma arises from fully differentiated epidermal melanocytes or melanocyte stem cells located in the bulge region of hair follicles, and the alternative theory, which proposes that multipotent dermal stem cells (DSCs), capable of differentiating into melanocytes, serve as the cellular origin of melanoma. UV-radiation, particularly UVA (320–400 nm), which penetrates deeply into the dermis, and UVB (280–320 nm), which affects the epidermis and the upper dermis, are known environmental carcinogens contributing to melanoma development. The multipotent DSCs are located in the dermis and thereby are potentially exposed to UVA and UVB-radiation. It is unclear if and how UV-radiation influences DSC differentiation into melanocytes, and what consequences pre-damaged DSCs will have for the differentiated melanocytes. This study explores the impact of UVA, UVB, and combined UVA+B irradiation on DSC differentiation into melanocytes. To this end, it is being investigated whether DSCs completely repair UV-induced DNA damage (cyclobutane pyrimidine dimers; CPD) before differentiation is initiated, or whether CPDs are still detectable during the differentiation process and in melanocytes.

**Methods:** DSCs were isolated from human foreskin tissue, cultured in stem cell medium and purified by using MACS® immunomagnetic cell sorting with NGFRp75 labeling, achieving stem cell frequencies exceeding 95%. Highly pure DSCs were cultured to 80–90% confluency and exposed to different single and multiple UV radiations. After 24 hours, cells were transferred to melanocyte differentiation medium and cultured for 12 days with medium changes every 2–3 days. Melanocyte differentiation was evaluated through immunostaining and quantitative PCR (qPCR) for melanocytic markers like MITF, TRP1, and HMB45. CPDs are detected with immunofluorescent staining at various times during the differentiation process. The analysis is carried out using a confocal laser scanning microscope (Leica Stellaris 5).

**Results:** Single UVA, UVB, and UVA+B irradiation of dermal stem cells (DSCs) induced morphological changes while not altering the expression of melanocyte-specific markers. These findings suggest that UV exposure does not alter the overall differentiation outcomes when compared to non-irradiated cells. Detailed analysis of differentiation state dependent CPD repair as well as the effects of multiple UV irradiations are under investigation and will be presented in our poster.
**Conclusion:** The data on repair of UV-induced DNA-damage (CPD) in dependence of differentiation state of the cell (from DSCs up to melanocytes) will give important information on the cell of origin of melanoma and thus will help to elucidate the mechanisms of melanoma development.

# Induction of DNA Damage by Far-UVC Radiation in Sensitive Human Skin

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**Keywords:** Far-UVC, 222 nm, vulnerable skin, DNA damage, CPD, radiation protection

The application of far-UVC (222 nm) is being discussed as a potentially safer alternative to conventional UVC (254 nm) due to its germicidal properties. During the SARS-CoV-2 pandemic, the use of far-UVC radiation for air disinfection increasingly came into focus as a potential solution for use in public spaces, even in the presence of people. However, further research is needed, particularly for sensitive or vulnerable populations, to assess potential risks.

In this study, for the first time, pediatric skin (<19 months; foreskin) and aged skin (>60 years) were analyzed under standardized conditions regarding the induction and localization of DNA damage (cyclobutane pyrimidine dimers, CPDs) following irradiation with far-UVC222 and UVC254.

The skin samples were irradiated with 30, 300, 1000, and 2000 J/m² of far-UVC222 and UVC254. CPD induction was analyzed immediately after irradiation using fluorescence immunohistochemical staining, microscopy, and quantification of CPD-positive nuclei in a layer-specific manner (basal and suprabasal layers of the epidermis). HaCaT keratinocytes were irradiated with 10, 30, and 50 J/m² far-UVC222 and 30 J/m² UVC254. HaCaT cells were stained for CPDs and γH2AX and analyzed using immunofluorescence microscopy.

The results clearly show that both UVC wavelengths induce DNA damage. Far-UVC222 causes significantly fewer CPDs compared to UVC254. Notably, in the basal cell layer, which is relevant for carcinogenic processes, no UV-induced CPD formation was observed after Far-UVC222 exposure, in contrast to UVC254.

At the same time, findings suggest that epidermal characteristics, such as stratum corneum and epidermal thickness, influence the sensitivity of different skin types. Additionally, the DNA-damaging effects of far-UVC through CPD and γH2AX induction are supported by investigations in cell culture.

These results highlight the importance of wavelength filtering and the targeted consideration of individual skin characteristics to further minimize potential risks, particularly for vulnerable groups such as children and the elderly. This project focuses on the mechanistic examination of DNA damage localization and provides a basis for future safety assessments of far-UVC.
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# The role of metabolites in the phototoxicity of sunscreens

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**Keywords:** oxybenzone, phototoxic properties, phosphorescence, laser flash photolysis.

The use of ultraviolet (UV) filters is now widespread for millions of people. However, some of them are not fully biodegradable, which has led to their diffusion and bioaccumulation in various ecosystems, causing a high environmental impact. These sunscreens or their metabolites are able to react in presence of light causing highly negative effects on the environment. This impact directly affects coral bleaching, among others.

Oxybenzone (**OB**) ($C_{14}H_{12}O_3$), derived from benzophenone, is a very common UV filter in sunscreens, but recent studies show that its absorption by certain marine organisms leads to its metabolization and the formation of phototoxic glycosides derivatives. In this work, two Phase II metabolites, **M1** ($C_{20}H_{22}O_8$) and **M2** ($C_{20}H_{20}O_9$), of **OB** have been synthesized and characterized in order to determine their photophysical and phototoxic properties.

The formation of the triplet state of the commercial product 2,4- dimethoxybenzophenone (**2,4**) and the metabolites **M1**and **M2**, has been monitored by phosphorescence and laser flash photolysis techniques. By means of the latter, the deactivating nature of $O_2$ has been verified through the decrease of the triplet state lifetime of the three compounds. In addition, the sensitizing nature of **2,4**, **M1** and **M2** has been observed through the different types of reaction classified in photobiology as Type I, II and II.

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# Sun protection by head wear

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**Keywords:** head wear; sun protection;

Hats, caps, and various head wear are commonly worn across the world recreationally, as safety gear (e.g. hard hats), for cultural reasons, or to protect from sun and heat.

In order to evaluate the effective protection of head wear of a variety of types and materials against Ultraviolet radiation (UVR), the erythemally effective UVR exposure was measured outdoors for more than 20 items of headwear under largely cloud-free conditions in May and June in Vienna, Austria. Measurements were taken by affixing UVR-meters of the type SunSaver to 21 body positions on a manikin placed upon a rotating platform. In order to simulate exposure during random directional walking, the platform was rotated at approximately one revolution per minute. Measurements with each piece of headwear were taken for five rotations and at three different solar heights between 20° and 65°. The protection factor of head wear (HwPF) was calculated as the ratio of measurements without head wear and with head wear. In addition, all items were evaluated in a laboratory setting to determine the Ultraviolet Protection Factor (UPF) of the respective material composition according to ISO standard protocol.

Results show that the top of the head is well protected by all items. As expected, protection on the nose, for example, varies from 2 up to 30 depending on type of hat and solar height. Lower protection (HwPF of up to 10) was found for other areas in the face for hats without face guard while face guards provide HwPF up to 50. Protection factor for the middle of the neck is generally less than 20 for hats with a full brim and minimal to none for most. The majority of head wear does not protect shoulders, torso, and limbs.
To sum up, head wear delivers appropriate sun protection of the head. The protection factor depends, among other factors, on the size of the brim. As only very large brims protect the shoulders to a certain extent, shoulders need additionally sun protection means like clothing or sunscreens.

# From seafood byproduct to solar cells: valorization of shrimp shell astaxanthin for renewable energy applications

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**Keywords:** DSSC; natural dyes; xanthophyll; de-esterification

Dye-sensitized solar cells (DSSCs) have gained attention as low-cost photovoltaic devices, with natural pigments serving as sustainable alternatives to synthetic sensitizers [1]. Among these, the carotenoid astaxanthin exhibits ideal photoelectrochemical properties for DSSC applications due to its broad visible light absorption and ability to anchor onto $TiO_2$ surfaces. This study investigates astaxanthin extracted from shrimp shell waste (Aristaeomorpha foliacea) [2] as a natural sensitizer, comparing its performance with purified astaxanthin standard.

An eco-friendly extraction process using ethyl acetate yielded a crude astaxanthin-rich extract containing predominantly esterified forms (93% of total carotenoids). When tested in DSSCs, the crude extract showed limited performance (PCE = 0.09%), attributed to poor interaction between esterified astaxanthin and $TiO_2$. Saponification converted these esters to free astaxanthin, significantly improving PCE to 0.30% by enabling bidentate coordination through liberated hydroxyl groups [3]. Electrochemical impedance spectroscopy confirmed reduced charge transfer resistance ($R_{CT}$) in saponified extracts (9.2 Ω cm² versus 16.4 Ω cm² for crude extract), approaching the performance of standard astaxanthin ($R_{CT}$ = 3.3 Ω cm², PCE = 0.45%). Notably, the saponified extract achieved 67% of the standard astaxanthin's efficiency, demonstrating shrimp waste as a viable alternative source. The remaining performance gap likely results from co-extracted lipids in the natural extract that may interfere with dye loading. These findings highlight the potential of seafood byproducts for solar energy applications while maintaining the advantages of natural sensitizers - low toxicity, renewability, and compatibility with green chemistry principles. Further optimization through purification or co-sensitization could bridge the performance gap with synthetic standards.

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# Bio-hybrid Devices Operating via Photosynthetic Bacteria

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Photosynthesis sustains life on planet Earth, transforming sunlight into energy. This ability is not limited to heterotrophic plants, but also to some bacteria that utilize light energy for their growth and development. One of the most extensively studied species is *Rhodobacter (R.) sphaeroides*, a Gram-negative, purple non-sulphur (PNS) bacterium known for its remarkable metabolic diversity. The crucial energy transduction process mainly depends on the bacterial reaction center (RC), a transmembrane multi-subunit protein able to convert sunlight into other chemical energy forms.

In this communication, the whole wild-type, metabolically-active photosynthetic bacterial cells of *R. sphaeroides*, and their carotenoid-less mutant strain, were integrated into a two-electrode architecture, to output a positive photovoltage upon illumination. Furthermore, Photosynthetic bacteria were also integrated into a light-electrolyte-gated organic transistor to produce a photomodulated electronic current, as well as in a biophotonic power cell working on direct sunlight. This proves that bio-organic hybrid optoelectronic devices may enable environmentally safe and cost-effective energy production.

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# Long-term toxicological impact of tattoo pigments

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**Keywords:** tattoo, phototoxicity, degradation, skin

The colored pigments used in tattooing are currently mostly organic compounds. Maintained in the dermis by macrophages, they are likely to undergo different degradation processes, including the impact of exposure to sunlight. We explored the hypothesis that these degradation phenomena could generate soluble products or small particles capable of migrating to the epidermis where they would participate in the log-term toxic effects of tattoos. Indeed, allergies and photosensitization phenomena in tattooed area have been documented. In addition, some studies suggest a link between tattooing and skin cancers.
In the present study, we assessed the *in vitro* cytotoxicity of a series of seven pure pigments, and focused our work on the three most toxic: pigment orange 13 (Po13), pigment red 254 (Pr254) and pigment red 122 (Pr122). Aqueous suspensions were aged by exposure to simulated sunlight at 40 ° C for 96 h. Particles were characterized and toxicological properties were determined. The presence of putative photoproducts was assessed by HPLC-mass spectrometry analysis. Photoproducts were also produced in larger amounts by irradiation on suspension in organic solvents.
The morphology and surface charge of the Po13 particles were little modified by aging, but their size was reduced. Soluble photoproducts were detected in the liquid fractions. The major photoproduct (DCBP) was produced in large quantities in suspension in isopropanol and purified. The toxicological profiles of the aged suspensions, their soluble fractions and DCBP were then determined on the HaCaT keratinocyte cell line. The impact of Po13 suspensions on viability was hardly affected by aging. In contrast, the soluble fractions were more toxic after photoaging. Suspensions and filtrates did not induce the release of reactive oxygen species or the formation of DNA strand breaks. The samples showed only limited effects on the proteome of HaCaT cells. Conversely, DCBP was cytotoxic and induced ROS production, but was not genotoxic. DCBP was found to activate CYP450 monooxygenases known to be involved in xenobiotic metabolism [1].
We applied the same approach to the study of two other red pigments: Pr122 and Pr254. Pr254 was very stable under the degradation conditions applied and did not lead to the formation of significant amounts of photoproducts. Photoaging did not modulate its cytotoxicity. Under its pristine form, Pr122 was the most toxic pigment investigated and this toxicity was hardly affected upon photoaging. Yet, some photoproducts could be isolated and partly characterized. Some of them were found to be cytotoxic.
Altogether, our results show that photodegradation of pigment particles may lead to the formation of soluble photoproducts and the release of diffusible nanoparticles. Further investigation in skin models or in human should thus be undertaken to determine the contribution of these processes to skin toxicity in tattoos.

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# 2-Mercaptonicotinoyl glycine prevents HEV-induced pigmentation

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**Keywords:** Hyperpigmentation; Visible Light; HEV light; Photoprotection

Hyperpigmentation and pigmentary disorders are major clinical consequences of sun exposure. While UV radiation is a known contributor, visible light (VL), particularly High Energy Violet (HEV) light (400-450nm), also induces long-lasting pigmentation, especially in melanocompetent individuals, Fitzpatrick Phototype III and above [1, 2]. It was recently shown that HEV can activate melanogenesis via the Opsin 3 photoreceptor [3] and is also able to induce reactive oxygen species and subsequent oxidative stress [4]. Given the contribution of VL to hyperpigmentation issues, photoprotection in this wavelength range is recommended. The efficient solutions rely on the use of pigments, absorbing and diffusing VL [5]. However, the tint and opacity of these products may not appeal to consumers. Actives which could prevent VL-induced pigmentation appears then of interest.

In this work we aimed at assessing 2-mercaptonicotinoyl glycine (2-MNG, 0.5 and 1%), as melanogenesis inhibitor [6], and Ascorbic acid 7%, as strong antioxidant, to counteract HEV-induced pigmentation, in 2 controlled randomized clinical trials, including in all 58 individuals with Fitzpatrick Phototype III or IV. These agents have already shown their efficiency in reducing UV-induced pigmentation [7]. Delineated areas on the subjects back, topically treated or not by the product, were exposed to HEV once a day for 4 days. The product was applied before, during and 5 weeks after HEV exposure. Pigmentation was assessed using chromametry and visual scoring. While Ascorbic acid did not exhibit any efficacy versus its vehicle in limiting skin pigmentation induced by HEV, the use of 2-MNG led to an early significant decrease in HEV-induced pigmentation, which was sustained until the end of the study. The effect of 2-MNG was evidenced by colorimetry, and was also visualized and significantly scored by the expert. Moreover, a 2-MNG dose effect could be evidenced at early time points.

These results highlight the potency of 2-MNG to reduce HEV-induced pigmentation, offering an alternative or a complement to tinted products.

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# Reversible Control of cAMP Activity in Living Cells by Visible Light

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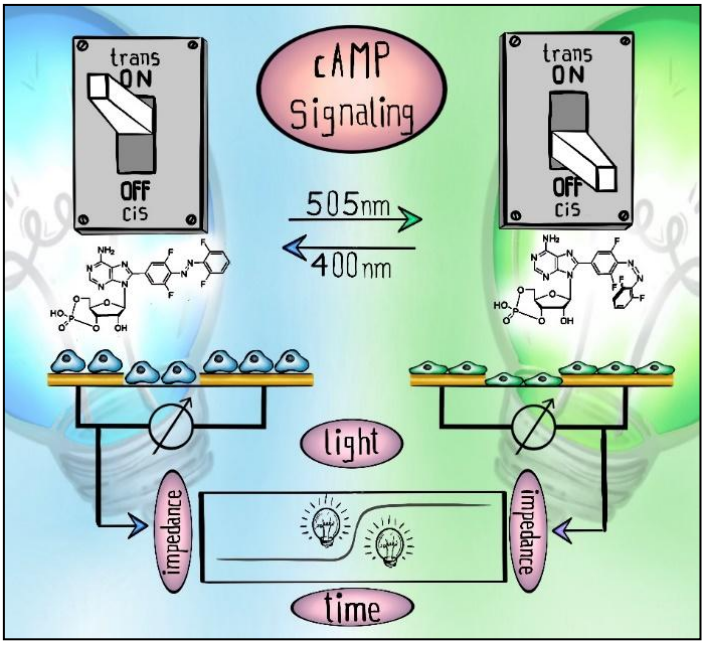
**Keywords:** cAMP; ECIS; Photochromism; Signal Transduction

Cyclic adenosine monophosphate (cAMP) is one of the most prominent molecules involved in intracellular signaling. As a second messenger, it regulates a plethora of biochemical processes that are essential to keep a cell alive and orchestrates physiological responses to external stimuli. Ever since its discovery, great efforts have been made to elucidate all the molecular mechanisms involved in cAMP-mediated signaling cascades. However, experimental evidence suggests that cAMP-mediated signal transduction is much more complex than previously assumed. For new insights, it is crucial to study the real-time dynamics and reversibility of the cAMP-related signaling mechanisms, which often remain disguised by classic pharmacological assays or modulators of cAMP signaling. By chemically attaching a photoswitchable moiety to cAMP, we got control over the biological activity of the molecule through visible light of different wavelengths. Combined with label-free electric cell-substrate impedance sensing (ECIS), the dynamic response of living cells treated with photochromic cAMP has been monitored in real time while the cAMP-mediated signaling cascade is reversibly switched *on* and *off* by visible light with high temporal resolution.

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# Photoregulation of interleukin-receptor interactions through genetically encoded photoresponsive non-canonical amino acids

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**Keywords:** protein-protein interactions (PPI), cytokines, genetically encoded non-canonical amino acids (ncAA), photocaged proteins, photoswitchable proteins.

## ABSTRACT

Cytokines play key roles modulating the immune and inflammatory responses. To date, several cytokines have been approved for cancer immunotherapy, e.g. interleukin-2 (IL-2). However, the medical application of cytokines is often hindered by toxicities and/or modest efficacies. Human interleukin 24 (IL-24) is an immunomodulatory cytokine that represents an important target for the treatment of autoimmune diseases and cancer. Thus, the spatiotemporal control of interleukin-receptor interactions may help to further expand the utility of these biomolecules as protein-based therapeutics.

Here, we report on a light-induced ON-switch IL-24/IL-20R2 heterodimer assembly based on genetically encoded photocaged and photoswitchable noncanonical amino acids. Using a combination of biophysical, molecular biology and cell-based assays, we show that photocaged non-canonical amino acids (nitrobenzyl-tyrosine [1], nitropiperonyl-lysine) introduced at critical positions of recombinant IL-24 or IL-20R2 dramatically reduce the binding affinity between the two. Mild irradiation with UV light removes the caging group thus enabling complex assembly and activation of the JAK/STAT signaling cascade. Similarly, a photoswitchable non-canonical amino acid (azobenzene-phenylalanine) provides a certain degree of control over interleukin-receptor interactions.

These results provide a proof-of-concept for the rational design of photoactivatable interleukin-receptor pairs with potential application in oncology, immunology and infection biology.

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# RIPK4 knockout enhances melatonin's anti-proliferative effect on melanoma cells

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**Keywords:** melanoma, melatonin, proliferation, RIPK4, spheroids

Despite significant advances in targeted therapy and immunotherapy, melanoma cells are becoming resistant, resulting in relapse. Consequently, there is a need to identify new therapeutic targets. One potential candidate appears to be receptor-interacting protein kinase 4 (RIPK4), a serine/threonine kinase that modulates multiple signaling cascades critical for tumorigenesis.

To date, the role of RIPK4 in melanoma is poorly understood. Thus, our studies suggest that RIPK4 may play an important role in the growth as well as progression of melanoma. This process, particular *in vivo* assessments, may be strongly regulated by microenvironmental factors. Herein, we propose melatonin, whose synthesis also occurs in cutaneous cells and which exerts anti-inflammatory as well as oncostatic effects, including on melanoma.

In this study, we assessed the effects of melatonin and RIPK4 knockout on melanoma cell viability and proliferative potency using both 2D and 3D cultures. We employed CRISPR/Cas9-mediated downregulation of RIPK4 in A375 and WM266.4 human melanoma cell lines, confirmed by Sanger sequencing and Western blot analysis. Cell viability was tested by MTT viability assay, proliferation by cyclin D1, as well as fluorescent labeling of Ki-67 proliferation marker. The 3D model was used to evaluate spheroid size and ATP metabolic activity.

Our results revealed that melatonin significantly reduced melanoma cell viability in a dose-dependent manner. Among the investigated cell lines, Sk-Mel-28, which exhibits the highest level of RIPK4 expression, has been found as the most sensitive cell to melatonin. Additionally, melatonin treatment significantly reduced the growth and metabolic activity of melanoma spheroids, with enhanced effects observed in RIPK4 knockout cells.

The results obtained in this study suggest that the use of melatonin and RIPK4 kinase knockout may have synergistic effects in terms of inhibition of melanoma cell growth, however, this requires further studies targeting preclinical models.

# Red Light Photobiomodulation Activates AMPK-Fatty Acid Oxidation and Enhance Mitochondrial Respiration in Keratinocytes

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## ABSTRACT

For over 60 years, photobiomodulation (PBM) has been studied for its promising results in various treatments, such as neurological diseases [1]. In recent years, these therapies gained growing scientific and public interest due to their demonstrated efficacy in skin rejuvenation, pain relief, anti-inflammatory effects, among other benefits [2]. While mitochondrial modulation appears central to PBM effects, the exact molecular mechanisms for its therapeutic actions remain unclear. To address this issue, we investigated mitochondrial function in keratinocytes following 660 nm red light exposure, by assessing respiratory dynamics via Oroboros-2K respirometry and conducted comprehensive metabolic flux analysis using the Seahorse XF platform. Irradiation with 660 nm LED enhanced keratinocyte proliferation while significantly increasing both ATP-linked and maximal mitochondrial oxygen consumption rates (OCR). Using Resipher OCR Monitoring System, we further demonstrated that this photobiomodulation effect sustained elevated basal OCR for up to 48 hours post-irradiation with no significant differences in electron transport chain complexes expression. OCR measurements in permeabilized cells had no effects by red light, suggesting the modulation of cytosolic metabolic processes. By evaluating metabolic fluxes supported by different substrates (glucose, amino acids and fatty acids), we observed that red light specifically activates oxygen consumption related to fatty acid oxidation (FAO), effect that was confirmed by the consumption of free fatty acids. Western blot analysis showed increased phosphorylation of AMPK and ACC proteins, indicating that red light activates AMPK, which inhibits ACC, thereby promoting β-oxidation. Since AMPK can be activated by CAMKK2—a calcium-dependent kinase, OCR was measured with $Ca^{2+}$ chelator (BAPTA-AM), showing decreased OCR, suggesting a role of $Ca^{2+}$ signaling and a possible CAMKK2 involvement. Overall, PBM in keratinocytes regulates mitochondrial respiration via allosteric mechanisms and $Ca^{2+}$ signaling. Solid knowledge on the PBM mechanisms will certainly open several opportunities in photomedicine.

**Keywords:** Photobiomodulation, Mitochondrial respiration, Metabolic flux, Regulatory Effects.

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# Nitric oxide-iron association enables identification of dinitrosyl iron complex, exhibits antioxidant properties and increased nitric oxide release by UVA in human keratinocytes cells

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UV radiation triggers the release of NO in an enzymatically independent manner in skin. During exposure to radiation, this process happens via release of NO stores such as nitrite and nitrosothiols (1). Dinitrosyl iron (DNIC) are complexes formed by iron, NO and glutathione, and when in the cell, can induce increase in citoprotection and antioxidant properties (2;3;4). These complexes have been identified in macrophages, liver and kidney tissue.(3,4) However, in the skin, the presence of such compounds is unknown. In this study we evaluated the ability of human keratinocytes (Hacat cells ) in form DNIC when treated with iron-NO association. The ability of this association in induce antioxidant profile in the cell and increase release of NO induced by UVA (365NM-25J) was also investigated. The tretament with Fe2+ plus NO-satured solution allow the identification of DNIC by EPR. The association between NO and iron decrease the fluorescence of CM-H2DCFDA probe, when compared with Fe2+ only, suggesting the antioxidant capacity related to NO-iron association. The evaluation of nitric oxide release with DAF-FM-DA probe, using cells treated with iron, shows that the treatment with iron increase NO release after the exposure to UVA radiation, which indicate the increase formation of NO stores related iron in the cell. Our results, shows that NO-iron association can allows DNIC identification, antioxidant properties and increased NO release in NO cells. These profile also suggests that NO-iron association can be a new type o NO stock that can respond to UVA radiation in the skin.

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# Blue Light and Retinal: Key Drivers of Lipofuscin Accumulation in Keratinocytes

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**Keywords:** Lipofuscin; Visible light; Retinal; Autophagy

Lipofuscin, often referred to as the 'aging pigment,' is known to produce reactive species capable of modifying biomolecules. This is exemplified by its role in generating mutagenic DNA lesions in keratinocytes and melanocytes through singlet oxygen ($^1O_2$)-mediated pathways upon visible light exposure[1]. A key component of lipofuscin in retinal pigment epithelial (RPE) cells, N-retinylidene-N-retinylethanolamine (A2E), originates from the visual cycle. During photoreceptor activity, the hydrolysis of the opsin-isomerized chromophore bond releases *all*-trans-retinal, which subsequently contributes to A2E formation[2]. Although the role of opsins and retinal in RPE lipofuscinogenesis is well known, this mechanism has not been investigated in skin cells, and the pathways leading to lipofuscin accumulation in these cells remain poorly understood. In this study, we used human immortalized keratinocytes (HaCaT). Cells were seeded in 6- or 48-well plates and cultured under standard conditions. At 50% confluence, they were treated for 30 minutes with either 5 µM *all*-trans-retinal (Retinal group) or vehicle (Control group), with all procedures carried out under light-protected conditions. Following incubation, cells were irradiated in PBS with 100 J/cm² of blue light (447 nm, irradiance: 21.22 mW/cm²; Irradiation group), while a parallel group was kept in the dark (Dark group). After treatment, the medium was replaced, and cells were maintained in culture until further analysis. Forty-eight hours after exposure, *all*-trans-retinal combined with irradiation increased lipofuscin accumulation, as detected by Sudan Black staining. This combination also reduced mitochondrial viability (MTT), without affecting lysosomal viability (NR). The Autophagy Arbitrary Units (AAU) index, derived from MTT, NR, and crystal violet assays, revealed that retinal plus blue light inhibited autophagic flux compared to the Dark control. Given the link between lipofuscin accumulation and impaired autophagy[3], we analyzed p62 and LC3-II immunocontent 24 hours post-irradiation. While p62 remained unchanged, LC3-II levels increased in irradiated groups, with bafilomycin A1 (Baf-A1) further elevating LC3-II in controls. To assess the potential for a visual cycle-like pathway in keratinocytes, we quantified the expression of OPN3, RDH8, and RDH12 four hours post-treatment. While RDH8 was undetectable in HaCaT cells, RDH12 was significantly upregulated in retinal-treated groups. This increase suggests that HaCaT cells possess the enzymatic capacity to convert *all*-trans-retinal into its less reactive reduced form, *all*-trans-retinol. However, if *all*-trans-retinal is not efficiently metabolized, it may accumulate and serve as a precursor for A2E and lipofuscin formation[4]. Although RDH12 was upregulated by retinal, RDH8 appears to be absent in these cells. Further investigation is needed to clarify how the low RDH8 expression affects retinal regeneration within the visual cycle in HaCaT cells. Notably, the Retinal-irradiated group exhibited increased OPN3 expression compared to the Dark Ctrl group. If a functional visual cycle exists in HaCaT cells, this suggests that *all*-trans-retinal could be isomerized to 11-cis-retinal, which in combination with blue light, may activate OPN3. This process could create a positive feedback loop that further upregulates OPN3 expression. Future studies should investigate the functional consequences of this activation, particularly its potential role in lipofuscin accumulation and autophagy modulation. Our findings underscore retinal's critical role in light-induced lipofuscinogenesis. As an endogenous photosensitizer, elucidating retinal's photochemistry in keratinocytes could pave the way for novel photoprotective strategies and advance our understanding of skin physiology.

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# Novel System to Produce Light-Induced Automatic Gap on cell culture plates: *in vitro* Wound Healing Assay

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**Keywords:** Wound healing assay; Photodynamic Therapy; Phototoxicity; Light system

*In vitro* cell culture migration assays, also commonly referred to as *Wound Healing Assays*, are a valuable tool to evaluate the effect of a treatment after an injury by the capacity of cells' motility to fill the damaged area (gap area). Traditional methods include the *Scratch Assay*, in which a pipette tip is used to produce a gap in the cell layer, and the commercial ibidi® Culture-Insert 2 Well, in which a silicone gasket is placed on top of the well/plate and cells are seeded on both chambers, creating a defined cell-free area between them [1]. While the first method has the advantage of being cheaper, requiring only a pipette tip and an operator, it is also highly dependent on the operator's performance, limiting reproducibility. This reproducibility is easily achieved with the ibidi® Culture-Insert 2 Well, which features a fixed gap area. However, it is also less representative of the physiological microenvironmental stress on cells when injury occurs, where cell edges are less defined and a "cell-free" area is rarely present. By taking advantage of photodynamic action, it is possible to produce a cell-free area by using a highly localized illumination of the cell layer [2].

Our new approach aimed to evaluate the capability of an automatic optical device - developed in-house - to replace traditional or commercial wound healing assay methods in routine laboratory settings. For this purpose, the device was used to irradiate culture plates containing human dermal fibroblasts (HDF) that had been pre-treated with 5,10,15,20-tetrakis(1-methylpyridinium-4-yl) porphyrin (TMPyP) as a photosensitizer (PS) under aerobic conditions, to create a defined gap area. Different concentrations of PS and cell culture densities were tested. The effectiveness of the device was assessed regarding the gap width produced in each culture well after irradiation. Optical microscopy cell culture photography was obtained, and fibroblast viability was assessed after 24 h. The advantages and limitations of this novel approach compared to the other methods will be discussed.

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# Molecular Dynamics simulations of Fucoxanthin and Chlorophyll a/c Binding Proteins of different Diatoms

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**Keywords:** Diatoms, FCPs, molecular dynamics, photoprotection, MSM

Diatoms are unicellular, photosynthetic, eukaryotic algae that live and thrive into marine ecosystems [1]. They are extremely important for life on Earth as they produce approximately 20% of the global oxygen supply [2]. Fucoxanthin and chlorophyll a/c binding proteins (FCPs) are their unique Light Harvesting Complexes (LHCs) which have the ability to absorb light in the blue-green region (450-550 nm) underwater. In addition to performing photosynthesis, diatoms exhibit a secondary mechanism that protects them from excessive light exposure [3]. This photoprotective or quenching mechanism converts the excess excitation energy into heat. During photoprotection, photosynthesis is downregulated, so as a result there is a reduction in oxygen production and biomass formation. Our study focuses on these unique organisms employing more than 100 μs molecular dynamics (MD) simulations of FCPs at all-atom resolution from two different organisms: *Phaeodactylum tricornutum* [3] and *Chaetoceros Neogracilis* [4]. *Classical and enhanced sampling* MD has been combined with Markov State Model (MSM) analysis to explore different conformations of the FCPs over long time scales. We compare the dynamics between monomeric, dimeric and tetrameric FCPs from the diatom *Ph. tricornutum* with monomeric and tetrameric FCPs from *Ch. Neogracilis* under different acclimation states (low-high light exposure). Remarkably, we identify similar dynamics of the FCP scaffold in response to light which can be associated with a common motif in the down-regulation of photosynthesis. By comparatively studying the (down) regulatory mechanisms of photosynthesis across different diatom species, it is possible to engineer diatoms that can quickly recover from photoprotection in order to increase and improve the production of biomass and atmospheric $CO_2$ assimilation (greenhouse gas). This can be achieved by creating chimeric complexes that combine features from different diatom species to minimize energy losses. Moreover, a deeper understanding of diatom photosynthesis could provide fundamental knowledge toward the development of artificial photosynthesis systems.

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# Novel implementation of single molecule optical tweezers in photosynthesis to uncover the conformational changes of the light-harvesting complexes regulating their energetics

**Martina Berglund**

Single molecule optical tweezers with correlated force-fluorescence measurements are a state-of-the-art technique that applies and measures forces on single proteins and, simultaneously, assess how the induced conformational changes affect the emission properties of the protein. This technique has never been applied to pigment-binding proteins and therefore neither to photosynthetic nor to any photosensory protein from the larger fields of photosynthesis and photobiology. We propose the use of optical tweezers to mechanically induce and measure conformational changes in photosynthetic light-harvesting complexes and correlate them, for the first time, to different energetic states of the LHCs within the same experiment - a multimodal approach previously unattainable. By applying this tool to the LHCs, we want to observe for the first time experimentally changes of structure and of emission at the level of single LHCs, as well as identify which protein domains are involved in the conformational change of the LHCs. I will here present this new approach and show our preliminary results on plant LHCs.

# Structural studies of phototropins – light receptors that control plant growth and development

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**Keywords:** UV/blue light, phototropins, *Arabidopsis thaliana*, photocycle

Phototropins are light-sensitive serine/threonine kinases that play a crucial role in blue and UV light perception and regulation of photomorphogenesis in plants. Their activity depends on conformational changes induced by light absorption in LOV (Light, Oxygen, Voltage) domains, which initiates a signaling pathway leading to physiological responses. *Arabidopsis* has two phototropins, namely phototropin1 and phototropin2, which are redundantly involved in regulating chloroplast movements induced by changes in blue light irradiance. The outcomes of cellular signal transduction may be associated with a change in phototropin homo and heterodimer formation. The purpose of our study is to understand the molecular structure of phototropins, with a particular focus on the mechanisms by which phototropins change their activity in response to light intensity. Here we present the general course of the experiment from expression and purification of recombinant phototropins, through data collection and structural analysis using Cryo Electron Microscopy (Cryo-EM). The results will provide important information on the performance of plant photoreceptors and may have applications in plant engineering and optogenetics.

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

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

## ABSTRACT

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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# Analysis of the chloroplast avoidance and kinetics of chloroplast movements in the forest-floor plants

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**Keywords:** chloroplast movements; forest-floor plants; photosynthesis.

Chloroplast movements are important to maintain photosynthetic sufficiency [1][2]. In their natural environments, plants received sunlight of varying intensities. For instance, forest-floor plants often experience rapid and irregular fluctuations in irradiance due to sunflecks—brief, intense bursts of sunlight that penetrate the canopy. In this study, we investigated chloroplast avoidance responses and the kinetics of chloroplast movement after the light is turned off in six forest-floor plant species: three dicots-*Vaccinium myrtillus*, *Hepatica nobilis*, and *Oxalis acetosella* and three monocots—*Maianthemum bifolium*, *Paris quadrifolia*, and *Convallaria majalis*. Our findings indicate that species exhibiting greater amplitudes of chloroplast avoidance also show faster movement kinetics. To assess whether these differences are related to leaf anatomy, we examined leaf cross-sections but found no consistent anatomical features that could explain the observed pattern. Interestingly, monocot species exhibited pronounced chloroplast movements, regardless of whether they have a clear differentiation of the mesophyll e.g., *Paris quadrifolia* or less pronounced differences between the palisade and spongy parenchyma. We also compared chloroplast avoidance in both spring and overwintered leaves of *Hepatica nobilis* and *Oxalis acetosella*. Spring leaves exhibited stronger chloroplast avoidance, which may be attributed to a difference in their dark transmittance. All the investigated species typically grow in shaded environments with low PAR levels, which do not promote chloroplast avoidance [3][4]. Therefore, the potential differences in photosynthetic parameters associated with varying chloroplast movements might be most apparent primarily under high light conditions, such as sunflecks, which stimulate the chloroplast avoidance response. Understanding these dynamics is important as it can provide insights into plants' adaptive strategies to fluctuating light and the ecological significance of chloroplast avoidance.

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