SIFB Italian Society of Photobiology

XXXV Annual Conference – Mediterranean Edition

Messina 9 – 11 September 2024

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Conference Program

Invited Talks	(25' + 5' discussion)
Keynote presentations	(20' + 5' discussion)
Oral presentations	(15' + 5' discussion)
Young investigators prizes	(20' + 5 discussion)
Flash presentations	(6')

Monday September 9th (morning session)	
8.15	Registration
9.15	Congress Opening and Welcome Remarks: Valentina Rapozzi (President SIFB), Antonino Mazzaglia (Chair Conference), CNR-ISMN and Dip. ChiBioFarAm (UNIME) Directors & SCI Sicilia President
Session 1 - Photosynthesis & photoresponsive materials Chair: Antonino Mazzaglia	
9.30	**Massimo Trotta** Photosynthetic Odyssey: Sustainable Solutions from a paramount metabolic process
10.00	**Rossella Labarile** Photosynthetic bacteria in light-driven bio hybrid devices
10.20	**Raffaella Margherita Zampieri** Biohydrogen production through photofermentation by a purple non-sulfur bacterium
10.40	COFFEE BREAK
Session 2 – Miscellaneous Chairs: Greta Varchi, Massimo Trotta	
11.15	**Masaoki Kawasumi** The effect of UV-induced mutations on the binding of ETS transcription factors to the Cdkn2a/p16 promoter
11.40	**Laura Grigolato** Daylight PDT: pros and cons
12.00	**Alberto Danielli** Nanobiological engineering of M13 phages for enhanced electrochemiluminescent signal transduction in point-of-care biosensors
12.20	**Simona Salerno** Chitosan-Porphyrin Composite Membrane for Topical Photodynamic Therapy
12.40-	**Sonia Visentin** Assessment of Cystic Fibrosis Mucus Permeability to Fluorescent Bacterial Secretome Molecules for Drug Candidate Potential
13.00	LUNCH

Monday September 9th (afternoon session)

Session 3 – Photodynamic inactivation & antimicrobial PDT

Chairs: Domenico Franco, Kristjan Plaetzer

14.10	**Kristjan Plaetzer** Save the Crop: Photodynamic Inactivation in Agriculture
14.35	**Bianka Siewert** Photoactive Antimicrobials from South American Fungi
14.55	**Mariachiara Trapani** A nanohybrid assembly composed of silver nanoparticles and porphyrins for antimicrobial photodynamic applications
15.15	**Nidia Maldonado-Carmona** Shaping light: photophysical and biological parameters needed for efficient Photodynamic Antimicrobial Chemotherapy on bacteria
15.35	**Giuseppe Nocito** Curcumin and Vancomycin loaded hydrogel coating medical device for prosthetic joint infections control
16.00	COFFEE BREAK

Session 4 – Photo-induced chemical and biological processes

Chairs: Carlo Matera, Masaoki Kawasumi

16.30	**Wiktor Szymanski** Photopharmacology: tools, considerations, and applications
17.00	**Rosalba Sortino** Three-photon infrared stimulation of endogenous neuroreceptors in vivo
17.20	**Edoardo Armano** Photoactivatable Version of Ivabradine Enables Light Induced Block of HCN Current in Vivo
17.40	**Lorenzo Torrisi** Carbon dots luminescence via carbon laser ablation in biocompatible solution
18.00	SIFB meeting

Tuesday September 10th (morning session)

Session 5 – Anticancer PDT

Chairs: Dmitri V. Krysko, Nela Malatesti

9.05	**Luis G. Arnaut** Tumor Priming and Photodynamic Therapy: A renewed hope for oncology
9.30	**Claudia Ferroni** Bioresponsive catalase-like nanoplatforms for combined photo/sono and immuno-therapies of hypoxic cancers
9.50	**Marzia Bruna Gariboldi** Polydopamine-Coated Liposomes for Methylene Blue Delivery in 2D and 3D Anticancer Photodynamic Therapy

10.10	**Francesca Bianco** Molecular mechanism of Br-SQ-C4 phototoxicity: the role of Ca^{2+} and ROS interplay
10.30	**Dmitri V. Krysko** Immunogenic Cell Death in Glioma Immunotherapy: Efficacy of Photodynamic Therapy and Dendritic Cell Vaccines
11.00	COFFEE BREAK

Session 6 – Anticancer PDT

Chairs: Marzia Gariboldi, Luis. G. Arnaut

11.30	**Angeles Juarranz** Role of squamous cell carcinoma microenvironment in the response to Photodynamic therapy
12.00	**Michela Nigro** Molecular engineering of a spheroid-penetrating phage nanovector for targeted photosonodynamic treatment of EGFR-expressing cancer cells
12.20	**Carlotta Pontremoli** Hafnium based metal–organic framework entrapping squaraines for efficient NIR-responsive photodynamic therapy against pancreatic cells
12.40	**Matteo Di Giosia** Bioconjugation and supramolecular interaction of proteins with photoactive agents for theranostic applications
13.00	**Flash presentations**
13.30	LUNCH & POSTER SESSION

Tuesday September 10th (afternoon session)

Session 7 – Photosensitizers' design & functionalization

Chairs: Enrico Caruso, Angela Scala

14.30	**Engin U. Akkaya** Targeted Endoperoxides for Precise Delivery of Singlet Oxygen
15.00	**Francesco Fagnani** Novel highly luminescent N^C^N-Pt(II) derivatives for bio-imaging and photodynamic therapy
15.20	**Vicente Marchán** Novel photosensitizers based on Ru-coumarin complexes for combating hypoxic tumors
15.40	**Ester D'Agostino** Design and synthesis of a glyco-porphyrin for the conjugation of bioactive peptides
16.00	**Mathias O. Senge** Molecular and Formulation Strategies for Photosensitizer Design
16.25	COFFEE BREAK

	Session 8 – Young investigators award ceremony Chair: Valentina Rapozzi
17.00	**Edoardo Jun Mattioli** Carrying Temoporfin with Human Serum Albumin: A New Perspective for Photodynamic Application in Head and Neck Cancer
17.25	**Paolo Emidio Costantini** A modular phage vector platform for targeted photodynamic therapy of Gram-negative bacterial pathogens
18.30	SOCIAL EVENT
21.00	SOCIAL DINNER

Wednesday September 11th (closing session)

Session 9 – Miscellaneous
Chair: Matteo Calvaresi, Mathias O. Senge

9.15	**José Ruiz** Achieving red-light anticancer photodynamic therapy under hypoxic conditions using Ir(III)-COUPY conjugates
9.40	**Matteo Calvaresi** Light-enhanced cytotoxicity of approved anticancer drugs
10.00	**Salvatore Mirabile** Light-up of 4-(1-piperazinyl)phenol derivatives targeting tyrosinase
10.20	**Luigi Donato** From NGS to Optogenetic Innovations Towards Retinal Channelopathies Therapies
10.45	COFFEE BREAK
	Session 10 – Miscellaneous Chairs: Maria Teresa Rossi, José Ruiz
11.15	**Nela Malatesti** Balancing the lipophilicity of tri(pyridinium-3-yl)porphyrins for improved entry into melanoma cells and photodynamic effect
11.40	**Martina Mušković** Synthesis, physicochemical properties and in vitro evaluation of amphiphilic pyridiniumporphyrins with N-oxide moiety for use in photodynamic therapy (PDT)
12.00	**Lucia Pappagallo** Neurophage: molecular engineering of phage nanoparticles for non-invasive neuronal photostimulation
12.20	**Giovanni Romano** Multicolor endoscopic source for intragastric phototherapy against Helicobacter pylori
12.40	**Giulia Neri** Appraising the Efficiency of NIR-Responsive Hybrid Nanosystem based on Graphene, Poly(methacrylic acid) and Gold Bipyramids in Melanoma Cancer Cells
13.00	CLOSING REMARKS

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Invited speakers

Engin U. Akkaya

Targeted Endoperoxides for Precise Delivery of Singlet Oxygen

Engin U. Akkaya[1,2]

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Singlet oxygen generated by photosensitization is the primary cytotoxic agent involved in photodynamic therapy (PDT) of cancer and a few other non-malignant indications. However, because of i) very limited light penetration in the 400-900 nm region, ii) very low tumor oxygenation, and iii) difficulty in standardization of a therapeutic protocol, PDT did not become a first line therapy, now more than a century after the experimental demonstration of the "photodynamic effect" and half a century after the first clinical trials.

In order to keep the potential benefits of PDT, while eliminating its inherent limitations, we posited that judicious delivery of chemically generated singlet oxygen at the right place, would open a new path for development of a singlet oxygen-based cancer treatment methodology.[1-3] This approach should not be confused with standard chemotherapeutic regimens, because the carrier/storage compound does not need to be toxic, and cytotoxicity would be limited to singlet oxygen, delivered on location.

With these considerations, we designed and synthesized naphthalene-derived endoperoxides[4] with mitochondria targeting triphenylphosphonium moieties.[5] Thermal release of singlet oxygen inside the mitochondria is very effective in causing cell death by induction of apoptosis, as evidenced by both 2D and 3D cell cultures. In vivo efficacy of the method was also studied using a mouse model and found to be very encouraging. Targeted delivery of singlet oxygen to cancer cell mitochondria could lead to the breakthrough needed to make better use of intentionally generated ROS in cancer therapy, without any need for external light, or depletion of already low tumor oxygen.

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Angeles Juarranz

Role of squamous cell carcinoma microenvironment in the response to Photodynamic therapy

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Cancer-associated fibroblasts (CAFs), an important component of tumor microenvironment, have gained prominence owing to their crucial role in the promotion and therapy resistance of squamous cell carcinoma (SCC) of skin and also of oral cavity. Among the approved therapies for the types of cancers is Photodynamic therapy (PDT). The main objective of this work is to develop functional three-dimensional models that simulate the characteristics of in situ and invasive solid tumors to be used as in vitro platforms in the study of the progression of skin and oral SCC, as well as in the response to photodynamic therapy (PDT). To this end, heterotypic spheroids have been generated using skin and tongue SCC cell lines and CAFs isolated from patients with SCC. CAFs were then characterized based on the expression of determined markers (GAL-1 and endoglin, α-SMA, vimentin) and related with the type of tumor they were originated. Confocal microscopy confirmed that the models met the characteristics of the arrangement of tumor cells and CAFs of *in situ* and invasive carcinomas. Furthermore, functional assays indicated that CAFs promoted the migration of cells expressing epithelial-mesenchymal transition (EMT) molecules (vimentin and N-cadherin) more than those that lack expression of these EMT markers. Migration capacity was also dependent on the type of CAF; those isolated from more aggressive SCC, promote cell migration to a greater extent in invasive models. In addition, we have observed that the transforming growth factor β1 (TGFβ1) cytokine secreted by CAFs drive resistance to PDT in the epithelial SCC cells in these models. The results obtained allow us to indicate that the generated models constitute an excellent tool for studying the role of CAFs in the migration and response to PDT of SCC cells being TGFβ1 CAF-derived a promising biomarker to predict the suitability of PDT.

Wiktor Szymanski

Photopharmacology: tools, considerations, and applications

Wiktor Szymanski[1,2]

[1] *Department of Medicinal Chemistry, Photopharmacology and Imaging, Groningen Research Institute of Pharmacy, University of Groningen*
[2] *Medical Imaging Center, University Medical Center Groningen, University of Groningen.*

Molecular photomedicine holds the promise for precise treatments, which avoid systemic adverse effects and development of drug resistance. This promise is supported by current medical imaging modalities that are able to reveal the nature and location of malignancies, such as cancer and infections. At the same time, biomedical engineering has recently created methods to deliver light deep into human body. The photomedicine puzzle is currently missing its final piece – the way of translating light into a therapy.
To address this challenge, drugs could be introduced whose activity could be reversibly or irreversibly turned on with light. The aim of this presentation is to describe the emerging concept of photopharmacology (Figure A),[1] which is currently being developed and applied to precisely control the activity of drugs using light. The presentation will focus on our efforts towards bridging light and medicine, focusing first on new light-operated tools[2] (molecular photoswitches[3,4,5] and photocages[6,7], Figure B). Next, I will highlight the synergies between medical imaging and therapy, offered by light, through photo-responsive optical[8] and magnetic resonance[9] imaging agents. The examples of light-controlled bioactive molecules presented will include small molecules[10,11] and proteins[12]. Finally, using those examples, I will highlight the structural aspects[13] of photopharmacology.

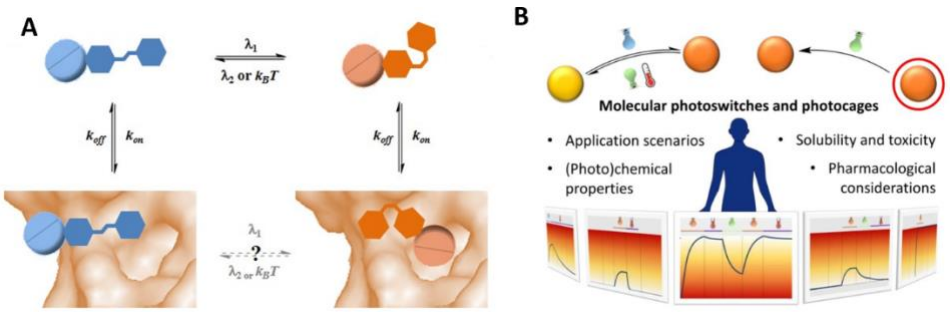

Figure. The principle of photopharmacology (A) and its key molecular tools (B).

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Massimo Trotta

Photosynthetic Odyssey: Sustainable Solutions from a paramount metabolic process.

Massimo Trotta

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The universe is a pretty big place. If it's just us, it seems like an awful waste of space [1]. And if we are not alone, what would be the common trait among inhabited planets? Most likely, it would be the natural energy sources available, such as the energy generated by thermonuclear reactions occurring in stars, and consequently, the natural energy conversion systems they possess. On our planet, photosynthetic organisms are the primary energy converters that sustain life on Earth for at least three billion years. The chances that this paramount process is general across the universe are very high.
How invaluable and universally relevant would it be to unlock and leverage the capabilities of photosynthetic organisms to set forth a positive and virtuous path? In this presentation, we will embark on a brief journey exploring the profound impacts that technologies based on photosynthesis, including artificial and semi-artificial methods, have already brought or are poised to bring to our planet [2]. Given our urgent need for sustainable and circular processes, these advancements hold great promise for future development.

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Young Investigator Award

Paolo Emidio Costantini

A modular phage vector platform for targeted photodynamic therapy of Gram-negative bacterial pathogens

Annapaola Petrosino [1], Roberto Saporetti [2], Francesco Starinieri [1], Edoardo Sarti [1], Luca Ulfo [1], Luca Boselli [3], Andrea Cantelli [4 5], Andrea Morini [1], Suleman Khan Zadran [1], Giampaolo Zuccheri [1 6], Zeno Pasquini [7], Matteo Di Giosia [2], Luca Prodi [2 6], Pier Paolo Pompa [3], Paolo Emidio Costantini [1], Matteo Calvaresi [2 6], Alberto Danielli [1 6]

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[5]IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy.
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Growing antibiotic resistance has encouraged the revival of phage-inspired antimicrobial approaches. On the other hand, photodynamic therapy (PDT) is considered a very promising research domain for the protection against infectious diseases. Yet, very few efforts have been made to combine the advantages of both approaches in a modular, retargetable platform. Here, we foster the M13 bacteriophage as a multifunctional scaffold, enabling the selective photodynamic killing of bacteria. We took advantage of the well-defined molecular biology of M13 to functionalize its capsid with hundreds of photo-activable Rose Bengal sensitizers and contemporarily target this light-triggerable nanobot to specific bacterial species by phage display of peptide targeting moieties fused to the minor coat protein pIII of the phage. Upon light irradiation of the specimen, the targeted killing of diverse Gram(-) pathogens occurred at subnanomolar concentrations of the phage vector. Our findings contribute to the development of antimicrobials based on targeted and triggerable phage-based nanobiotherapeutics.

Edoardo Jun Mattioli

Carrying Temoporfin with Human Serum Albumin: A New Perspective for Photodynamic Application in Head and Neck Cancer.

Edoardo Jun Mattioli[1], Luca Ulfo[2], Alessia Marconi[1], Valentina Pellicioni[3], Paolo Emidio Costantini[2], Tainah Dorina Marforio[1], Matteo Di Giosia[1], Alberto Danielli[2], Carmela Fimognari[3], Eleonora Turrini[3], Matteo Calvaresi[1]

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[3]Dipartimento di Scienze per la Qualità della Vita, Alma Mater Studiorum—Università di Bologna, Corso d'Augusto 237, 47921 Rimini, Italy.

Temoporfin (mTHPC) is approved in Europe for the photodynamic treatment of head and neck squamous cell carcinoma (HNSCC). Although it has a promising profile, its lipophilic character hampers the full exploitation of its potential due to high tendency of aggregation and a reduced ROS generation that compromise photodynamic therapy (PDT) efficacy. Moreover, for its clinical administration, mTHPC requires the presence of ethanol and propylene glycol as solvents, often causing adverse effects in the site of injection.
We explored the efficiency of a new mTHPC formulation that uses human serum albumin (HSA) to disperse the photosensitizer in solution (mTHPC@HSA), investigating its anticancer potential in two HNSCC cell lines. Through a comprehensive characterization, we demonstrated that mTHPC@HSA is stable in physiological environment, does not aggregate, and is extremely efficient in PDT performance, due to its high singlet oxygen generation and the high dispersion as monomolecular form in HSA. This is supported by the computational identification of the specific binding pocket of mTHPC in HSA. Moreover, mTHPC@HSA-PDT induces cytotoxicity in both HNSCC cell lines, increasing intracellular ROS generation.[1] Applying a reverse docking approach, we also identified several blood transport proteins able to bind and disperse monomolecularly mTHPC. We validated the computational results synthesizing the mTHPC-apomyoglobin complex and assessing its PDT efficacy *in vitro*.[2]
We are currently work on the use of these hybrid systems in photochemotherapy (PCT), an emerging cancer treatment that combines phototherapy and chemotherapy. This combination therapy can be more potent than using either treatment alone, leading to additive and synergistic antitumor effects. Since PCT involves administering a photosensitizer (PS) and a chemotherapeutic drug, issues can arise due to the different pharmacokinetic and/or pharmacodynamic profiles of the two molecules.[3]
Using proteins as a platform for the simultaneous transport of both the PS and the chemotherapeutic drug represents as innovative approach to PCT. Through a virtual screening, we now identified chemotherapeutic drugs capable of binding to HSA in a different pocket than mTHPC, creating an efficient protein-based delivery systems loaded with both the chemotherapeutic agent and mTHPC.

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Keynote Speakers

Luis G. Arnaut

Tumor Priming and Photodynamic Therapy: A renewed hope for oncology.

Luis G. Arnaut [1]

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The tumor microenvironment is a major barrier to the success of therapies targeting cancer cells. The enhanced permeability and retention effect, associated with large gaps between endothelial cells in tumor blood vessels and poor lymphatic drainage, encouraged the use of nanostructures in tumor drug delivery. However, it is now increasingly evident that hyperproliferation of cancer cells increases the so-called "solid stress" in solid tumors, and that leakage from tumor blood vessels leads to elevated interstitial fluid pressure that hinder drug penetration into tumors. Aliviation of solid stress and of interstitial fluid pressure enables better tumor response to treatments and is a promising component of tumor priming strategies.

This presentation will discuss tumor priming strategies using light, either mediated by photoacoustic or photodynamic effects [1]. It is shown that the photoacoustic effect generated when relatively low fluence pulses (<50 mJ/cm^2) are absorbed by efficient light-to-pressure transducer materials, peak pressures of 100 bar are generated and increase drug penetration in tumors. It is also shown that the photodynamic effect using photosensitizers with light absorption in red/near-infrared, also increase the response of tumors to therapies, namely to immunotherapies.

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Masaoki Kawasumi

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

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Skin cancer is strongly associated with ultraviolet (UV) radiation that generates many mutations. There is thus a need to investigate what mutations drive skin cancer. Silencing of the *CDKN2A* gene, which encodes the p16 tumor suppressor protein, plays a key role in cancer progression. Our previous mouse study showed that chronic UV irradiation to skin induced mutations in the regulatory and gene body regions of tumor suppressor genes, including the *Cdkn2a/p16* promoter. Importantly, topical application of caffeine to mouse back skin reduced the frequency of these *Cdkn2a/p16* promoter mutations and suppressed skin cancer development. However, the impact of these promoter mutations on cancer progression remained unclear. We hypothesized that these mutations may inhibit the binding of critical transcription factors, thereby reducing the expression of the p16 tumor suppressor. The mutations at the *Cdkn2a/p16* promoter were found in the DNA sequence that was similar to the ETS transcription factor-binding element (EBE). To determine the functionality of this element (p16-EBE), we cloned this putative EBE into the reporter construct in which the binding of transcription factors to p16-EBE drives luciferase expression. ETS1 and ETS2 represent the ETS transcription factor family, with the latter expressed dominantly in skin. We found that overexpression of either mouse ETS1 or ETS2 resulted in higher luciferase activities than the corresponding control vector, indicating that ETS proteins bind to p16-EBE. We also tested mutated p16-EBE that carries the UV-induced mutation found in mice. With overexpression of mouse ETS1 or ETS2, the mutated p16-EBE showed markedly lower luciferase activities than wild-type counterpart, implying that the UV-induced mutation in p16-EBE inhibits the binding of ETS proteins to the *Cdkn2a/p16* promoter. This study highlights the importance of loss-of-function mutations in promoters that may contribute to cancer progression.

Dmitri V. Krysko

Immunogenic Cell Death in Glioma Immunotherapy: Efficacy of Photodynamic Therapy and Dendritic Cell Vaccines

Dmitri V. Krysko

Cell Death Investigation and Therapy (CDIT) Laboratory, Anatomy and Embryology Unit, Department of Human Structure and Repair, Faculty of Medicine and Health Sciences

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

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

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

Acknowledgments

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Nela Malatesti

Balancing the lipophilicity of tri(pyridinium-3-yl)porphyrins for improved entry into melanoma cells and photodynamic effect.

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Photodynamic therapy (PDT) for the treatment of tumours has numerous advantages over other therapies as it uses a molecule – a photosensitiser (PS) - that is non-toxic but has good absorption in the visible part of the EM spectrum and can transfer the absorbed photon energy to molecular oxygen, creating highly reactive singlet oxygen ($^1O_2$). For effective PDT, it is important that the PS is delivered effectively, that it accumulates in the tumor tissue and enters the cells, and that it generates $^1O_2$ as close as possible to the cell structures whose damage leads to cell death. The distribution and passive selectivity of PS can be enhanced by a balance between the lipophilicity of the molecule and the arrangement of the hydrophilic and hydrophobic parts of the molecule [1]. In the search for an ideal hydrophobicity-hydrophilicity balance, we have focused on amphiphilic AB_3 *meso*-tetraarylporphyrins that we prepare by quaternising the nitrogen of three pyrid-3-yl groups, while an alkyl chain (R) is attached to the fourth group, the amidophenyl group, to provide lipophilicity.

In our previous work, tri(pyridinium-3-yl)porphyrin with a long lipophilic chain (R = $C_{17}H_{35}$) showed a much stronger PDT effect on HeLa cells than hydrophilic porphyrin (R = CH_3) under low fluence red light conditions [2]. The presence of a long chain also proved to be the most important factor when comparing different pyridyl groups (3- *vs* 4-pyridyl) and the quaternisation of the pyridyl nitrogen atom (*N*-methylated *vs N*-oxidised) [3]. Therefore, we decided to carry out a systematic study with the mentioned AB_3 porphyrins, both with free bases and with zinc(II) complexes, and to compare compounds with different alkyl chain lengths (R = C_nH_{2n+1}, n = 7, 9, 11, 13, 15 and 17) to determine the most ideal photosensitiser in terms of possible aggregation, (photo)stability, $^1O_2$/ROS production and, above all, cellular uptake and localisation, and photodynamic activity on melanoma cell lines (MeWo and A375) and human dermal fibroblasts (HDF). Two wavelengths were used for photoactivation, red light at 645 nm and orange light at 606 nm, both with low fluence of light (2 mW/cm^2) for 30 min. Although zinc(II) complexes were shown to enter cells more slowly than free base porphyrins, phototoxicity was similar for both groups of photosensitisers at 606 nm and increased with increasing chain length. All porphyrins with the longest chain (n = 17) had the strongest activity, which was similar on all three cell lines tested, while the porphyrins with n = 13 appeared to be PDT efficient, but also showed selectivity, with significantly stronger activity on both melanoma cell lines compared to HDF.

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Kristjan Plaetzer

Save the Crop: Photodynamic Inactivation in Agriculture.

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Access to healthy, safe and sufficient food represents a fundamental human right. Bacteria, fungi and pest insects trouble growers by inducing plant diseases or by spoiling crops thus impeding harvest yields. Application of pesticides including antibiotics in agriculture has a negative impact on the environment and fires development of resistance. Photodynamic Inactivation (PDI) is a powerful tool to kill microorganisms. The aim of this study is to demonstrate that PDI is applicable to fight microbial pathogens and pest insects in agriculture. Formulations of sodium-magnesium-chlorophyllin (Chl, approved as EU food additive E140) serve as economic and eco-friendly photoactive substances and are tested to fight bacterial and fungal plant pathogens as well as *Drosophila melanogaster* and *Drosophila suzukii* as model systems for insect pests. Depending on the application, concentrations of 100 µM to 2 mM of Chl followed by illumination with blue LED light (395 nm) or sunlight in the range of 26.6 to 100 J/cm^2 induce a clear antimicrobial effect against plant pathogens such as *Erwinia amylovora* [1] or *Botrytis cinerea* [2], irrespective of their resistance against conventional treatment and without harming host plants, as proven using *Fragaria vesca* as model system [3]. The same experimental approach is applicable for fighting *Drosophila* fruit flies if the photosensitizer is either offered as food with 3% sucrose or sprayed onto insects. After a drug to light interval of eight hours and illumination with LED light (395 nm, 157.8 J/cm^2) all fruit flies can be killed. When using sun light (532.7 J/cm^2) for Chl activation up to 98.5% of *Drosophila melanogaster* are erased [4]. In conclusion, PDI based on formulations of Chl can be applied in agriculture to fight plant diseases and pest insects.

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José Ruiz

Achieving red-light anticancer photodynamic therapy under hypoxic conditions using Ir(III)-COUPY conjugates

José Ruiz[1], Enrique Ortega-Forte[1], Anna Rovira[2], Cormac Hally[3], Mireia Jordà-Redondo[3], Gloria Vigueras[1], Manel Bosch[4], Pezhman Ashoo[1], Santi Nonell[3], Vicente Marchán[2]

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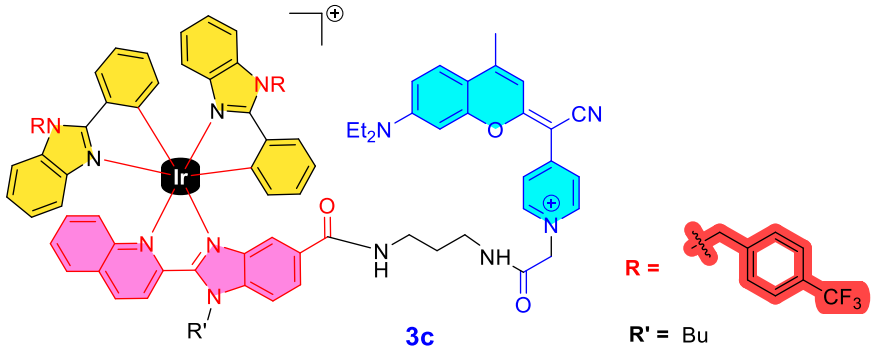
Despite the potential of photodynamic therapy (PDT), this oxygen-dependent cancer treatment is greatly restricted in the clinic by the well-known hypoxic feature of solid tumors. Here we provide new insights on the development of PDT agents based on conjugates between COUPY fluorophores and cyclometalated iridium(III) complexes with the aim of overcoming this limitation [1]. The structural modifications carried out within the metal core of Ir(III)-COUPY conjugates, based on the incorporation of trifluorobenzyl groups at the cyclometalating ligands, allowed efficient explotation of Type I PDT mechanisms while retaining operatiblity at long-wavelenght light, which facilitates deeper tissue penetration compared with short wavelenghts.
Photobiological evaluation revealed that the new Ir(III) COUPY conjugate, **3c**, achieved potent photocytotoxicity towards cisplatin-resistant A2780cis cancer cells, efficiently photogenerated ROS and photoinduced apoptotic cell death using red light irradiation. Interestingly, this Ir(III)-COUPY dyad retained such photoactivity under low-oxygen environments conditions, delivering equipotent photocytotoxicity towards normoxic and hypoxic adherent cancer cells.



Structure of the compound investigated in this work.

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Mathias O. Senge

Molecular and Formulation Strategies for Photosensitizer Design.

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A clinically useful photosensitizer must have the appropriate photophysical characteristics, allow for control of the relevant parameters via external stimuli, have a pharmacological profile appropriate for the target disease, and, ideally, elicit an immune response [1]. From a molecular design perspective, it would be ideal if some or all these criteria could be imprinted into a small molecule drug without having to resort to complex multimodality systems or multicomponent formulations. The presentation will give an overview about contemporary synthetic organic approaches in photosensitizer design focusing on the role of molecular shape and specific functional groups in improving PDT efficacy. Synthetic organic chemistry methods were used to prepare: a) BODIPY systems capable of excited state triplet formation via charge-separated states [2]; b) BODIPY and porphyrins capable of binding and releasing singlet oxygen [3]; c) atropisomerically pure photosensitizers and strapped porphyrins as mimics thereof with enhanced cellular uptake [4,5]; d) *m*THPC (Temoporfin), BODIPY, and protoporphyrin derivatives with functional groups suitable for linking to hydrogels [6]. Photophysical measurements were performed to establish the ground and excited state properties of the novel photosensitizers, while *in vitro* cell biological (melanoma and colon carcinoma cells) and *in vivo* (mice) studies were used to investigate the PDT efficacy of the novel systems. Rational planning of the molecular structure and reactivity in a synthetic molecular engineering approach was used to establish novel photophysical activation pathways for photosensitizers and to enhance cellular uptake and tissue targeting in PDT. While it is still difficult to design an optimal photosensitizer, the various design criteria outlined herein are suitable to improve many properties of light-activated drugs.

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Oral Communications

Edoardo Armano

Photoactivatable Version of Ivabradine Enables Light Induced Block of HCN Current in Vivo.

Edoardo Armano[1], Alessandro Porro[2], Federico Brandalise[2], Rebecca Appiani[1], Monica Beltrame[2], Andrea Saponaro[3], Clelia Dallanoce[1], Koichi Nakajo[4], Kaei Ryu[4], Roberta Leone[2], Gerhard Thiel[5], Marco Pallavicini[1], Anna Moroni[2], Cristiano Bolchi[1]

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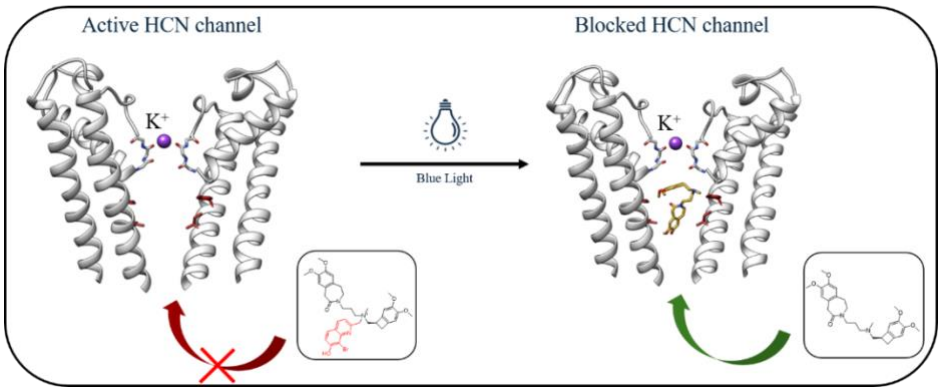
Therapeutic drugs that are temporarily inactivated by a photoremovable protecting group (PPG) offer the advantage of precisely targeting diseased tissues both spatially and temporally, thereby enhancing their bioavailability and effectiveness. Photoremovable protecting groups (PPGs) are small molecules that, when bound to a bioactive compound (forming a caged compound), release it through irradiation with a specific wavelength, allowing spatial and temporal control over its activity. PPGs also allow for easier tuning of the physicochemical and pharmacokinetic properties without affecting the biological activity of the active molecule.[1] In this research, we applied this method to ivabradine (IVA), a bradycardic medication used for angina pectoris and heart failure that specifically blocks HCN channels.[2] To address the side effects caused by its lack of selectivity among HCN channel subtypes (HCN1-4), we developed a caged version of IVA, linked to a photocleavable bromo-quinolinylmethyl group (BHQ-IVA). We demonstrate that exposure to blue light (440 nm) activates BHQ-IVA, releasing IVA, which then blocks HCN channel currents in vitro and produces a bradycardic effect in vivo. Both BHQ-IVA and the caging group are inactive on their own. The caged ivabradine remains stable in aqueous solutions and in the dark, and it retains its solubility and cell permeability, which are critical for IVA's activity. This strategy overcomes the poor subtype specificity of IVA, expanding its potential use to HCN-related diseases beyond cardiac conditions.



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Francesca Bianco

Molecular mechanism of Br-SQ-C4 phototoxicity: the role of Ca^{2+} and ROS interplay.

Francesca Bianco[1,3], Alessandra Gilardino[1], Giorgia Chinigò[1], Carlotta Pontremoli[2], Nadia Barbero[2], Alessandra Fiorio Pla[1]

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Photodynamic therapy (PDT) is a widely used treatment for both oncologic and non-oncologic diseases. It is based on the administration of a photosensitizer (PS) whose photoactivation induces cellular death due to the interplay of reactive oxygen species (ROS) and Ca^{2+} [1]. Among the different types of PS, the Polymethine dyes (PMD) are promising, due to their biocompatibility, phototoxicity and their absorbance in red-near infrared region of the light spectrum [2]. We characterized Br-SQ-C4 in order to investigate the intracellular signaling downstream its photoactivation using MCF-7 cell line. Cells have been incubated with the Br-SQ-C4 [1µM] overnight and then irradiated with RED-LEDs (λ=640 nm; Fluency: 3.84 J/cm^2) for 8 minutes. Beside phototoxicity, cytosolic, endoplasmic reticulum (ER) and mitochondrial Ca^{2+} signals as well as ROS release have been assessed using different fluorescent probes. We clearly demonstrated that Br-SQ-C4 localizes intracellularly at the level of the endoplasmic reticulum. We identified $O_2^{-\bullet}$ and $^{\bullet}OH$ as the main ROS mediators of photo-induced Br-SQ-C4 by using a system of ROS scavengers. Br-SQ-C4 photoactivation induces cytosolic Ca^{2+} increase due to Ca^{2+} entry from the extracellular medium as well as Ca^{2+} released from the ER which is followed by uptake by the mitochondria. Moreover, we demonstrated that ER Ca^{2+} release is a key player in light induced ROS production which in turn regulates ER Ca^{2+} release and the consequent mitochondria Ca^{2+} uptake. Future studies will better identify the role of Ca^{2+} channels in the signal transduction induced by Br-SQ-C4 photoactivation, and their potential role as target to increase the therapeutic efficiency of PS application in PDT.

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Matteo Calvaresi

Light-enhanced cytotoxicity of approved anticancer drugs.

Edoardo Jun Mattioli[1], Giulia Greco[2], Eleonora Turrini[2], Alessia Marconi[1], Paolo Emidio Costantini[3], Matteo Di Giosia[1], Alberto Danielli[3], Carmela Fimognari[2], Matteo Calvaresi[1]

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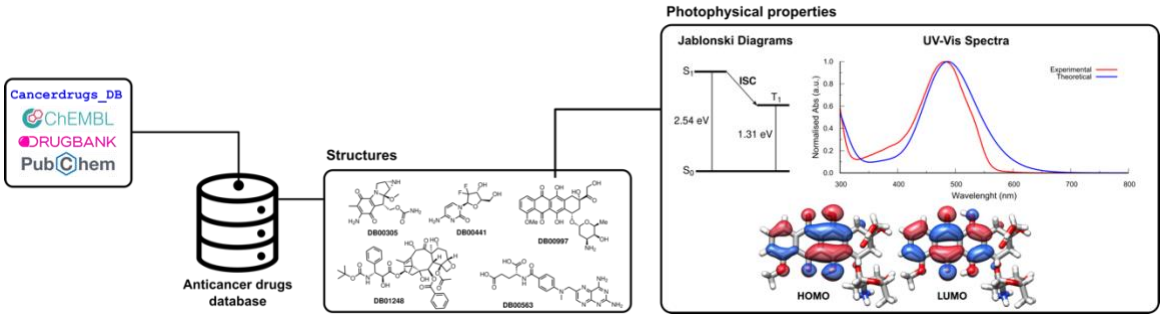
Photochemotherapy (PCT) is an emerging cancer treatment that combines phototherapy and chemotherapy [1]. This kind of combination therapy can be more effective than using either treatment alone, leading to additive and synergistic antitumor effects. Since PCT is based on the administration of two different compounds, a photosensitizer (PS) and a chemotherapeutic drug, many problems can arise due to the different pharmacokinetic and/or pharmacodynamic profiles of the two molecules [1]. Here we propose an innovative approach to PCT: the exploitation of the intrinsic photosensitizing properties of some chemotherapeutic drugs to enhance their anticancer activity upon light irradiation. Doxorubicin has already shown an enhanced anticancer activity upon irradiation [2]. Other approved anticancer drugs can potentially boost their cytotoxic activity through light activation.
We i) built a structural database of small molecules approved anticancer drugs, ii) calculated all the photophysical properties of these molecules, and iii) identified 17 promising molecules (i.e. mitoxantrone, pixantrone, daunorubicin, epirubicin) with the photochemical characteristics to be also a PS.



In principle, these chemotherapeutic drugs can be also used as photosensitizers in phototherapy. We selected the most promising candidates for *in vitro* testing. Photoactivation of the identified compounds generates peroxides and $^1O_2$ in a light-dependent manner and leads to an increase in their cytotoxic activity upon irradiation (reduction of the IC_{50} value up to an order of magnitude), triggering different modalities of cell death.

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Ester D'Agostino

Design and synthesis of a glyco-porphyrin for the conjugation of bioactive peptides

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Porphyrins are heterocyclic macrocycles, representing important prosthetic groups involved in different biological processes such as electron transfer (cytochromes), oxygen transport mechanisms (hemoglobin and myoglobin), or light absorption (chlorophyll). Porphyrins' derivatives have been deeply investigated and they can find applications in nanomaterials, bioimaging, and photodynamic therapy (PDT).[1] Recently, glyco-conjugation or peptide-conjugation has been performed to improve the biocompatibility and the cell penetration of porphyrin-based photosensitizers and probes, and these strategies are potentially useful for the refinement of the delivery of these systems and to reduce their toxicity.[2] When conjugated to a peptide, porphyrin groups confer different properties to the system (luminescence, photoresponsivity, catalytic activity), together with the ability to coordinate metals and produce ROS.[3] Recently, we synthesized, through a synthetic route involving cross-coupling Sonogashira reactions, an amphiphilic porphyrin functionalized in the meso position with a β-D-glucoside terminated rigid oligophenylene ethynylene (OPE), able to self-arrange into nano-aggregates in polar solvents and currently under study in its monomeric form as PS in photodynamic therapy (PDT).[4] Another strategy to obtain a glyco-conjugated porphyrin will be reported in this communication, involving a stereoselective method, passing through the formation of a galacto-dipyrromethene for the final obtainment of a trans-digalacto-functionalized porphyrin. Since polyfluorinated aromatic compounds readily react with thiolates through aromatic nucleophilic substitution (S_NAr) and provide excellent scaffolds for peptide conjugation, both the dipyrromethene and porphyrin synthesis have been synthesized starting from pentafluorinated aldehydes. The *in silico* design, the synthesis of a functional peptide and of a 5,15-bis-p-O-Galacto-(tetrafluorophenil)-10,20-bis(Pentafluorophenil)porphyrin, that can be used as a functional platform for regioselective stapling of peptides, will be reported. The galactose's moieties are employed to improve the hydrophilicity and biocompatibility of the porphyrin platform, while the peptide's moiety can be modulated to efficiently interact with biological targets.

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Alberto Danielli

Nanobiological engineering of M13 phages for enhanced electrochemiluminescent signal transduction in point-of-care biosensors

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Electrochemiluminescence (ECL) is widely used in bioanalytics, offering superior signal-to-noise ratios for biomarker detection, including nucleic acids, proteins, cells and viruses. Common ECL biosensors employ antibody sandwich assays, combining capturing elements and transducer antibodies labeled with ECL-active dyes. Bacteriophages can be conjugated with a high payload of photoactive molecules and engineered to display diverse targeting moieties on their capsids, providing a convenient platform for the development of innovative biosensors.
Here, we present the implementation of a phage-based biosensor allowing fast whole virion detection in ECL. M13 phages were genetically engineered to display a nanobody specific for the receptor binding domain (RBD) of the SARS-CoV-2 spike protein, in fusion with the minor coat protein pIII. The retargeting of the phage towards the SARS-CoV-2 Spike protein was demonstrated in both flow cytometry and surface plasmon resonance (SPR). The capsids of the newly generated $M13_{S1}$ virions were then chemically conjugated with hundreds of ECL dyes. Transducer phage targeting after conjugation with ECL dyes was validated in flow cytometry, taking advantage of the intrinsic fluorescence of the dyes, as well as in ECL where a strong signal enhancement was observed in comparison to antibodies, allowing attomolar detection of SARS-CoV-2 virions in less than an hour. This work demonstrates that in comparison to antibodies, phage-based ECL transducer elements provide enhanced sensitivity, reduced analysis time and production costs, while enabling portability and ease of use by non-specialists, potentially eliminating the need for more cumbersome (PCR) amplification strategies.

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Matteo Di Giosia

Bioconjugation and supramolecular interaction of proteins with photoactive agents for theranostic applications

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Photodynamic therapy (PDT) and photothermal therapy (PTT) are innovative approaches to treating various medical conditions, such as cancer or infections. Both therapies utilize harmless VIS-NIR light to activate a photosensitizing agent (PS) and achieve therapeutic effects, but they differ in their mechanisms of action. PDT is based on the local generation of reactive oxygen species (ROS) while PTT induces localized hyperthermia.
Photosensitizers may either be a single molecule species or a nanomaterial. Due to their hydrophobicity, most photosensitizers lack solubility and biocompatibility in the physiological environment, hampering the full exploitation of their potential in nanomedicine.
To address this issue, protein-based theranostics nanoplatforms were recently proposed for transporting hydrophobic photosensitizers. Due to their biocompatibility, physiological stability, structural diversity, and abundance from natural sources, proteins meet the strict requirements for biomedical usage. [1]
Human serum albumin (HSA) is the natural carrier of many hydrophobic endogenous and exogenous compounds. We demonstrated the possibility of using albumins and other proteins as versatile carriers for photosensitizers, both via covalent conjugation and supramolecular interactions, for PDT (i.e., Chlorin e6, Temoporfin, fullerenes) [2-5] and PTT (gold nanoparticles, carbon nanotubes) applications. We also developed alternative protein-based formulations, synthesizing protein nanoparticles. Spectroscopic and thermometric analysis showed that the photophysical properties of PSs were preserved upon protein binding. In addition, the functionality of the proteins can be improved with the bioconjugation of imaging tags and targeting agents.
The PSs-protein hybrids were tested in vitro and in vivo, evaluating their performances in cancer, antimicrobial, and regenerative medicine applications.

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Luigi Donato

From NGS to Optogenetic Innovations Towards Retinal Channelopathies Therapies.

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Inherited retinal dystrophies (IRDs) encompass a diverse array of genetic disorders that lead to progressive retinal degeneration and vision loss. While numerous causative genes for IRDs have been identified, the complex genotype-phenotype correlations often remain elusive. This study delves into the role of ion channel-encoding and related genes as potential modifiers of retinal dystrophies and explores their potential as targets for innovative optogenetic therapies.
Using Whole Exome Sequencing (WES) data from seven families, we identified variants in known IRD-related genes and conducted a comprehensive analysis of ion channel-related genes. Our findings suggest that mutations in ion channel-encoding genes, such as *CACNA1A*, *CHRNA7*, *CLIC5*, and *CNGB3*, contribute to the phenotypic variability observed in retinal dystrophies. These mutations impact ion channel structure, assembly, and function, ultimately affecting retinal signal transmission and processing [1].
Structural analyses then revealed significant alterations in the binding sites and stability of these mutated ion channels, implicating a direct impact on synaptic transmission and retinal cell function. Pathway enrichment analyses highlighted the involvement of these genes in critical processes like membrane depolarization, neurotransmitter receptor internalization, and response to light stimuli.
Our research also included a transcriptomic analysis using Total RNA-Seq, which provided insights into the effects of ion channel activation or inhibition in retinal pigment epithelium (RPE) cells. This approach focused on signal transmission, exocytosis regulation, and turnover, offering a comprehensive understanding of the genomic and transcriptomic landscape of retinal dystrophies [2].
Additionally, we are planning to develop optogenetic approaches to replace defective ion channels with light-sensitive proteins. These therapies will offer precise control over neuronal activity, potentially combined with targeted delivery methods for enhanced efficacy.
Preliminary data from patient genotyping demonstrate successful identification of genetic mutations responsible for retinal channelopathies. These findings highlight the potential of optogenetics in restoring function in patients with retinal channelopathies and pave the way for advanced therapeutic interventions..

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Francesco Fagnani

Novel highly luminescent N^C^N-Pt(II) derivatives for bio-imaging and photodynamic therapy

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Platinum(II) complexes having a terdentate 1,3-di(2-pyridyl)benzene (N^C^N) ligand present fascinating luminescent properties, useful for electroluminescent devices, sensors, bioimaging and photodynamic therapy (Figure) [1-2]. The structure of the complex can be functionalized in different ways, *e.g.* by inserting moieties on the pyridine rings and by varying the ancillary ligand on the metal center. As a consequence, also the photophysical properties of the compounds can be tuned on the basis of the structural modification. Up to now many substituents, both aliphatic and aromatic, have been tested on the central benzene ring and on the pyridines, leading to increased luminescence Quantum Yields (up to 100%) and more in general to improved photophysical properties. Furthermore, the replacement of the chloride ligand on the platinum can be a useful method to furtherly functionalize the molecule through the reaction with suitable species; examples can be the exploitation of a click reaction between an azide on the Pt and an alkyne (to give a triazole) [3], and of an isothiocyanate or a maleimide with thiolates such as cysteine residues. Such modifications open the way for different applications in the biological field, since complexes of this family could represent a potential tool for photoactivated chemotherapy, thanks to both the presence of an azido ligand, allowing for the anchoring to biological systems, and a platinum(II) center able to generate singlet oxygen when appropriately irradiated. This contribution aims to show the latest results in the investigation of functionalized N^C^N-Pt(II) complexes having remarkable luminescent properties and applications in photodynamic therapy.

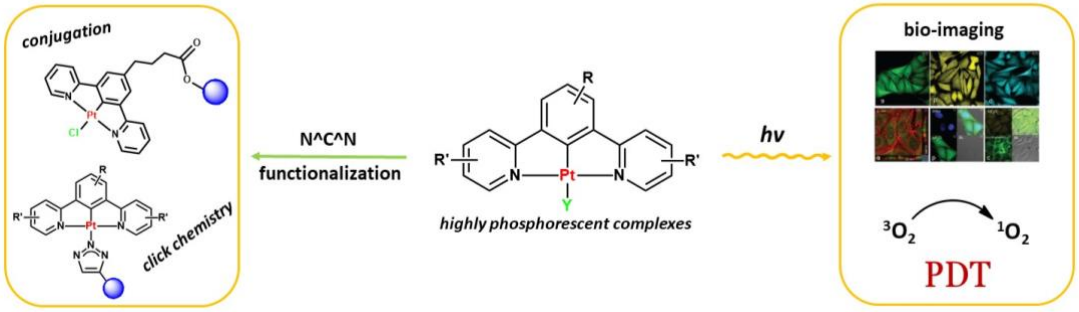

Figure. General structure of the N^C^N-Pt(II) complexes, with possible functionalizations and applications in biological field.

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Claudia Ferroni

Bioresponsive catalase-like nanoplatforms for combined photo/sono and immuno-therapies of hypoxic cancers.

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In recent years, activating the host immune system has emerged as promising oncological approach aimed at reversing tumor-mediated immunosuppression [1]. The immunosuppressive enzyme indoleamine 2,3-dioxygenase-1 (IDO1) proved to be highly expressed in the tumor microenvironment of many human solid tumors, representing a main driver in cancer immune escape [2,3]. Several IDO1 small-molecules inhibitors have been developed, and NLG919 has shown encouraging efficacy in combination with other anticancer approaches [4]. Among these, photo- and sono- dynamic therapies (PDT and SDT) represent a class of minimally invasive treatment modalities that, by combining non-toxic sensitizers, external stimuli (light or ultrasounds) and oxygen, produces cytotoxic reactive oxygen species, leading to apoptotic or necrotic cell death [5]. Giving the intrinsic immunoregulatory potential of PDT/SDT, their combination with immunotherapy has the potential to stimulate the host immune system and induce an abscopal effect [6]. However, the concomitant use of several drugs, characterized by different pharmacokinetic properties, bioavailability, and therapeutic concentrations, significantly hampers the success of multimodal treatments.
This study describes the preparation and characterization of higlhy stable albumin-based nanoformulations designed for the controlled and combined delivery of Nlg, as bioresponsive prodrug, and the sensitizer Rose Bengal (RB) for anticancer treatment with immunogenic cell death potential. Since the extreme hypoxia of the tumor microenvironment could restrict PDT/SDT effectiveness, catalase-like nanozyme has been encapsulated in the nanocarrier, favouring *in situ* oxygen production and enhancing the treatment efficacy. Nanoparticles have been characterized in terms of particles size distribution, polydispersity and z-potential. *In vitro* uptake and cytotoxicity were assessed in a panel of cancer cell lines, revealing that all cancer cells internalize nanoparticles and that, upon light exposure, our nanoformulation reduced cell viability, inhibited IDO1 enzyme, as well as triggered the release of damage-associated molecular patterns (DAMPs).

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Marzia Bruna Gariboldi

Polydopamine-Coated Liposomes for Methylene Blue Delivery in 2D and 3D Anticancer Photodynamic Therapy.

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Photodynamic therapy (PDT) is a relatively new therapeutic approach to cancer treatment in which, under light irradiation at specific wavelengths, photosensitizer molecules (PSs) react with molecular oxygen and generate reactive oxygen species, ultimately killing cancer cells [1]. Direct killing of tumor cells, vasculature damage induction, and stimulation of the immune system are the interrelated mechanisms involved in the antitumor effects of PDT [2]. Several conventional cell death pathways are implicated in the specific mechanisms through which PDT induces cell death, mainly apoptosis, necrosis, and autophagy [3]. Furthermore, non-conventional cell death modalities, such as pyroptosis, necroptosis, and ferroptosis, have been related to PDT-cytotoxic effects [4]. First- and second-generation PSs presented with problems that hindered their efficacy, such as photosensitization, low solubility, and selectivity. Third-generation PSs, consisting of second-generation PSs conjugated with targeting moieties or loaded into nanoparticles, are currently being developed to enhance PS cellular uptake and therapeutic efficacy [5]. Among other compounds investigated, the dye methylene blue (**MB**) showed potential as a PS and its photodynamic activity in tumor cells was reported even in its nanocarrier-delivered form, including liposomes [6].
In a previous work, we demonstrated that polydopamine (PDA)-coated liposomes can efficiently adsorb **MB** due to favorable electrostatic interactions [7]. Here the resulting **lipoPDA@MB** vesicles were first physico-chemically characterized and studies on their light stability and the in vitro release of **MB** were performed. The photodynamic effects of the vesicles were then assessed in a panel of monolayer- and 3D-cultured cancer cell lines, comparing the results with those obtained by free **MB**. **lipoPDA@MB** uptake, type of cell death induced, and the ability to generate ROS were also investigated. Our results showed that **lipoPDA@MB** possesses higher photodynamic potency than **MB** in both 2D and 3D cell models, likely due to its enhanced uptake, ROS production, and induction of apoptotic cell death. Therefore, **lipoPDA@MB** vesicles appear to be an efficient drug delivery system for **MB**-based PDT.

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Laura Grigolato

Daylight PDT: pros and cons.

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Daylight photodynamic therapy (dPDT) uses sunlight as an alternative source of irradiation and is employed in the treatment of actinic keratosis and other skin conditions. It started being used in Europe in 2006. Unlike conventional photodynamic therapy (cPDT), which requires three hours of incubation once the photosensitizer is applied, dPDT requires only 30 minutes. This short incubation time is due to the constant low-level activation of protoporphyrin IX (PpIX) during sun exposure. In cPDT, about 80% of PpIX is activated simultaneously within a few minutes of irradiation at a dose of 10 J/cm². In dPDT, however, the continuous activation of PpIX matches its formation rate, preventing its accumulation in the skin and resulting in reduced pain intensity compared to cPDT during treatment. In dPDT, sunlight (wavelength 380-700 nm) is used, differing from ultraviolet light (wavelength 100-380 nm), which is why the application of a sunscreen to block broad-spectrum ultraviolet light is recommended to prevent UV damage during sun exposure. dPDT is also more convenient as it can be performed by patients at home, and larger skin areas can be treated compared to conventional photodynamic therapy.
The effectiveness in treating actinic keratosis is similar in both methods. However, there are some limitations to dPDT, including the difficulty of scheduling sessions due to climatic conditions, especially in certain geographical areas. Another issue is the lack of strict control over the procedure. This paper addresses the main topics concerning this procedure to better understand its applicability and execution methods.

Rossella Labarile

Photosynthetic bacteria in light-driven bio hybrid devices

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Exploiting the abundant and renewable solar energy is crucial for transitioning to a low-carbon future. Solar power technologies offer immense potential to meet the rising global energy demand in a sustainable way. However, to maximize the potential of solar energy conversion, is essential to innovate and develop new materials that able to efficiently capture sunlight and convert it into energy forms. Among these materials, photosynthetic bacteria are a promising avenue due to their unique photometabolic properties. *Rhodobacter (R.) sphaeroides*, now reclassified as *Cereibacter sphaeroides* [1, 2], belonging to photosynthetic purple non-sulfur bacterium, exhibits remarkable potential for bioenergy applications.
We investigated the feasibility of developing biohybrid photoelectrochemical systems using polydopamine (PDA) [3]. Initially, we assessed the biocompatibility of PDA and dopamine, its monomer, through *in vivo* addition in the growth medium of *R. sphaeroides* under anoxygenic conditions [4]. Subsequently, we employed PDA conductive coatings to establish biotic-abiotic interfaces in biohybrid photoelectrochemical devices. These coatings encapsulated entire bacterial cells or specific components, such as the photosynthetic reaction center [5] of *R. sphaeroides* [6] and *R. capsulatus* [7]. This encapsulation facilitated electronic communication between the biological component and the electrode surfaces within the photoelectrochemical cells. Electrode modifications were also employed to produce more sustainable materials.
Furthermore, we observed measurable photocurrents at these bio-hybrid interfaces, demonstrating the efficacy of the approach.
In conclusion, our work highlights the potential of harnessing photosynthetic bacteria and polydopamine-based coatings to develop efficient and environmentally friendly solar-driven biohybrid systems for energy productions.

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Nidia Maldonado-Carmona

Shaping light: photophysical and biological parameters needed for efficient Photodynamic Antimicrobial Chemotherapy on bacteria

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Light-driven disinfection of pathogenic bacteria and fungi promises efficient results, without the appearance of antimicrobial resistance, as it is currently found with current chemotherapies. Photodynamic Antimicrobial Chemotherapy (PACT) relies on the use of light, oxygen and a photosensitizer (PS) agent, which upon light excitation is able to produce short-lived Reactive Oxygen Species (ROS), as result of electron transfer (Type-1 mechanism) or energy transfer (Type-2 mechanism). ROS are able to oxidize several biological targets, which decreases the likeliness to develop resistance to the treatment, while limiting the time that the PS is biologically active. The interest in this field has grown in the last decades, with most works devoting themselves to deliver exogenous PSs (i.e., porphyrins, phthalocyanines, chlorins and others) into the desired target to disinfect. However, delivering PS is not the only issue that scientists face when developing this technology. Light delivery entrails technical difficulties, specially if needed to be delivered inside corporal cavities (i.e., lungs, stomach, etc.) [1], and researchers should take in account the physical nature of the light (i.e., light source, emission wavelength, irradiance, irradiation time and light dose) and its behaviour and distribution in the desired target, taking in account light dispersion, diffraction and scattering, as well as temperature changes as consequence of the irradiation process. Thus, PACT needs the constant participation and collaboration of scientists from different field (i.e., physicists, biologists and chemists) and the miscommunication between the different parts, has sometimes led to confusing results in the literature, with results and experimental conditions difficult to replicate [2]. These lack of homogeinity in the results presented by the PACT community undermine the trust from the clinical community, which may impact the implementation of PACT in the clinical environment.

In the present work, we explored two different approaches commonly presented in light-driven disinfection publications. In the first one, we explored Rose Bengal disinfection, a classical photosensitizer, on a bacterial planktonic culture model. We explored the effect of different irradiances and light power delivered to bacterial survival and recovery, and we explored different ways to express the photodynamic effect and their biological meaning, comparing them with traditional approaches (counting surviving bacteria), but which result time and resources consuming. Furthermore, traditional models are normally end-point approaches, where the recovery of surviving cells after the irradiation process is usually overlooked.

Additionally, we considered the use of antimicrobial blue light (aBL) to explore the disinfection of bacterial biofilm. aBL is a photodynamic approach were endogenous PSs produce by bacteria are used, avoiding the need to deliver external photosensitizers into pathogenic bacteria. Bacterial biofilm usually grows attached to solid surfaces, and then the efficiency of aBL is affected by the geometry of the surface where the biofilm is grown. Thus, we are currently exploring how the geometry of the light irradiated (i.e., irradiance, covered surface, light penetration) and the irradiated surface (i.e., biofilm topography and geometry of the irradiated surface) need to be considered in regards to photodynamic efficiency, again, considering the biofilm recovery as an important biological aspect that needs to be taken care of [3]. Here, we present the model and photphysical parameters considered, while also presenting preliminary results of our model.

Together, we hope to mark a point on guidelines and considerations needed for PACT development, aiming to provide high quality results which can be extrapolated to the clinical environment in the near future.

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Vicente Marchán

Novel photosensitizers based on Ru-coumarin complexes for combating hypoxic tumors

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Photodynamic therapy (PDT) is a well-established, non-invasive modality for the destruction of tumors and/or tumor vasculature, based on the combination of three components, namely: a photosensitizer (PS), light of suitable wavelength, and oxygen. However, PDT is less efficient in the treatment of deep-seated hypoxic tumors, because it is an oxygen-dependent process. Here we introduce a new family of PSs based on Ru-coumarin complexes that take advantage of the well- established anticancer properties of transition metal complexes and of the rich and tunable photophysical properties of organic fluorophores. Phototoxicity studies have revealed that the PSs are non-toxic in the dark but become highly phototoxic when irradiated at different wavelengths within the phototherapeutic window. In general, the new PSs exhibited IC50 values in the very low nM range (e.g., 7.4 nM at 645 nm) and impressive PI values (PI > 34000) after deep-red light irradiation. In addition, the PSs display a good phototoxicity profile with highly penetrating NIR light (e.g. 760 nm) and retain an excellent photoactivity under hypoxia. Moreover, the PSs are aqueous-soluble, highly photostable and can be prepared in high purity from straightforward syntheses, which are also highly desirable attributes for further preclinical development. *In vivo* studies with one lead compound have demonstrated a good biodistribution profile, excellent safety and the ability to reduce tumor growth in a murine subcutaneous colorectal cancer model. Thus, the newly developed PSs are promising candidates for treatment of deep-seated hypoxic tumors, which is the cornerstone of current PDT technique.[1].

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Salvatore Mirabile

Light-up of 4-(1-piperazinyl)phenol derivatives targeting tyrosinase.

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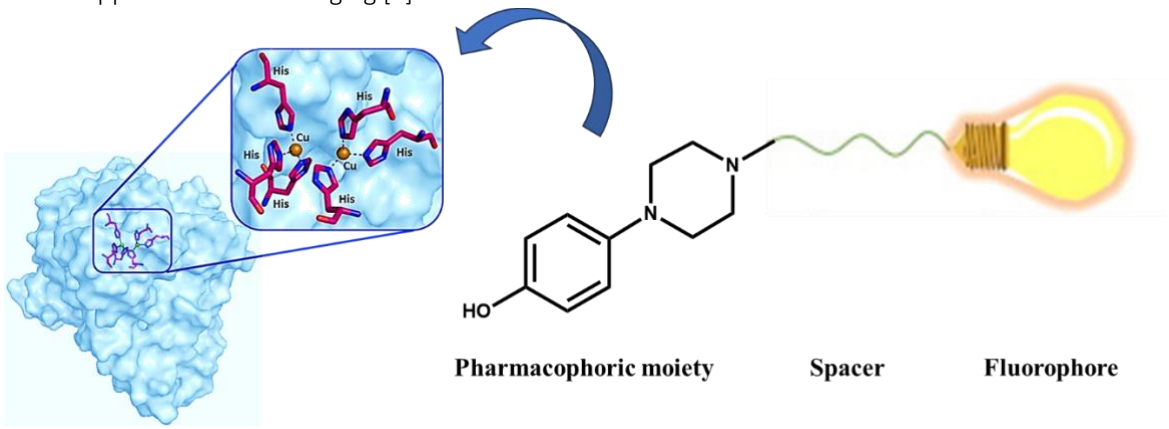
Tyrosinase (TYR, EC 1.14.18.1) is a copper-containing enzyme, that catalyzes the hydroxylation of phenols to catechol derivatives (i.e., tyrosine to L-DOPA), followed by further oxidation to the corresponding *o*-quinone products. It plays a prominent role in controlling the melanin biosynthetic pathway in distinct organisms. It has been established that the abnormal increase of TYR activity in melanocites is responsible for different hyper-pigmentation disorders and melanoma in humans. In addition, TYR is involved in dopamine neurotoxicity associated with Parkinson's disease. Therefore, a selective assessment of TYR as biochemical marker might be valuable for providing effective diagnostic/therapeutic approaches in pharmaceutical and cosmetic field. Furthermore, the chance to follow the TYR activities through the use of fluorescent probes has recently attracted considerable attention due to the possible applications in bioimaging [1].



In previous studies, we reported that the 4-(1-piperazinyl)phenol building block and its derivatives are substrate-like inhibitors, exhibiting TYR inhibitory properties reaching IC_{50} at low micromolar range against human and mushroom isoenzymes. By means of docking simulations, we hypothesized that, similarly to the natural substrate, the hydroxylphenyl substituent of these TYR inhibitors is located close to the two copper ions in the catalityc site [2-3]. Overall, these results inspired us the design and synthesis of further analogs bearing the 4-(1-piperazinyl)phenol moiety linked to fluorescent probes (e.g., BODIPY, curcumin and porphyrin derivatives). Each compound was tested in a spectrophotometric assay against TYR from *Agaricus bisporus,* as surrogate of human TYR that is generally used in preliminary *in vitro* testing

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Martina Mušković

Synthesis, physicochemical properties and in vitro evaluation of amphiphilic pyridiniumporphyrins with N-oxide moiety for use in photodynamic therapy (PDT)

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Photodynamic therapy (PDT) has proven to be an efficient antitumor therapy with few side effects and a low potential for activating resistance mechanisms. However, scientists are still searching for an ideal photoenzitizer that is highly efficient against tumor cell lines and could potentially overcome the disadvantages of the therapy, such as hypoxia. The use of *N*-oxide substituents in the porphyrin structure as a moiety of hypoxia-activated prodrugs (HAPs) is a strategy to overcome hypoxia and potentially activate other cytotoxic mechanisms. HAPs activated by hypoxia undergo reduction of two electrons leading to the formation of free electrons and/or active molecules that can then react with DNA or proteins [1].
Our group has previously shown that amphiphilic porphyrins with a long alkyl chain and quaternized pyridinium groups are efficient PSs, possibly due to their increased cellular uptake. Both *N*-methylated and *N*-oxidized pyridinium pophyrins conjugated with a long alkyl chain (17C) were highly photototoxic against various tumour cell lines (HeLa, MCF-7, HCT116 and HepG2), whereas the *N*-oxidized porphyrin (TOPyP3-$C_{17}H_{35}$) showed significantly lower phototoxicity on HFF cell line, which could be beneficial in PDT [2].
Here we present our study of a group of amphiphilic porphyrins bearing an alkyl chain of different lengths (9C, 13C and 17C atoms) with *N*-oxidized pyridinium-3-yl groups to be used for PDT. Their properties are evaluated using spectroscopic methods such as laser pulse photolysis (LFP) and time-correlated single photon counting (TC-SPC). In addition, singlet oxygen production by photodegradation of DPBF and the lipophilicity of the molecules are determined. The impact of the chain length was investigated in *in vitro* experiments by analysing cellular uptake, localization of PS and cytotoxicity in melanoma cell lines (MeWo and A375) and fibroblasts under conditions of normoxia and $CoCl_2$-induced hypoxia. The results are compared with *N*-methylated analogues conjugated to a chain of the same length and with hydrophilic analogues of both *N*-methylated and *N*-oxidized porphyrins.

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Giulia Neri

Appraising the Efficiency of NIR-Responsive Hybrid Nanosystem based on Graphene, Poly(methacrylic acid) and Gold Bipyramids in Melanoma Cancer Cells

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Cancer represents the second leading cause of human death worldwide, and it was estimated that 13 million people will die in 2030 [1]. In contrast to conventional methods, photothermal therapy (PTT) has emerged as an innovative, safe, efficient, and minimally invasive approach for tumor treatment. It employs an external laser source to irradiate targeted materials with high photothermal conversion efficiencies [2]. Thus, several photothermal agents characterized by string NIR absorbance, the well-known biological transparency window, are developed [3]. Among them carbon-based nanomaterials (CBNs) and gold nanosystems showed great potentiality in this field [3].
In this perspective we developed a novel NIR-responsive hybrid nanosystem (G-PMA@AuBPs) based on the combination of reduced graphene oxide (G), poly(methacrylic acid) (PMA) and gold bipyramids (AuBPs). To reach a high NIR absorbance G was selected, and derivatized with PMA improving its water colloidal stability. Subsequently, G-PMA system was functionalized with AuBPs, characterized by strong absorbance in NIR. The structure, morphology and chemical composition of hybrid materials was investigated by several techniques. The biocompatibility of G-PMA@AuBPs was assessed by *in vitro* studies, while its photothermal capacity was first examined in solution on B16F10 cells, employing a continuous-wave diode laser operating at 808 nm as the light source.
The obtained results showed the potentiality of G-PMA@AuBPs for PTT applications.

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Michela Nigro

Molecular engineering of a spheroid-penetrating phage nanovector for targeted photosonodynamic treatment of EGFR-expressing cancer cells

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Photodynamic (PDT) is a promising non-invasive approach for cancer treatment. This study aimed to enhance PDT effectiveness by engineering M13 bacteriophages as targeted vectors for efficient photodynamic killing of cancer cells. M13 phages were genetically engineered to display a nanobody, enabling specific recognition of the Epidermal Growth Factor Receptor (EGFR), overexpressed in various malignancies with severe outcome. The capsid of retargeted $M13_{EGFR}$ phages was further chemically functionalized with fluorophores or Rose Bengal (RB) sensitizers, demonstrating specific targeting to an EGFR-overexpressing A431 cell line, while showing minimal binding to EGFR-negative controls. The photodynamic performance of the modified phages was validated in vitro on both 2D and 3D cell culture models. Notably, in 3D spheroid models, the phages successfully penetrated the spheroid core, indicating their potential to target and treat cancer cells within complex tumor microenvironments. Remarkably, these modified phages exhibited potent and selective photodynamic killing activity at picomolar concentrations of the phage vector, upon irradiation with a white low power LED lamp. To assess translational potential, a yellow LED laser with higher irradiance was tested in vivo on a murine model (xenograft). Preliminary results demonstrated promising outcomes, validating the potential of this technology in a pre-clinical setting. Moreover, $M13_{EGFR}$ is able to bind also the EGFRVIII, a splicing variant of EGFR, present on glioblastoma cancer cells. These findings promote engineered M13 phages as promising nanovector platform for targeted photosensitization, paving the way to innovative approaches to fight difficult-to-treat malignancies.

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Giuseppe Nocito

Curcumin and Vancomycin loaded hydrogel coating medical device for prosthetic joint infections control

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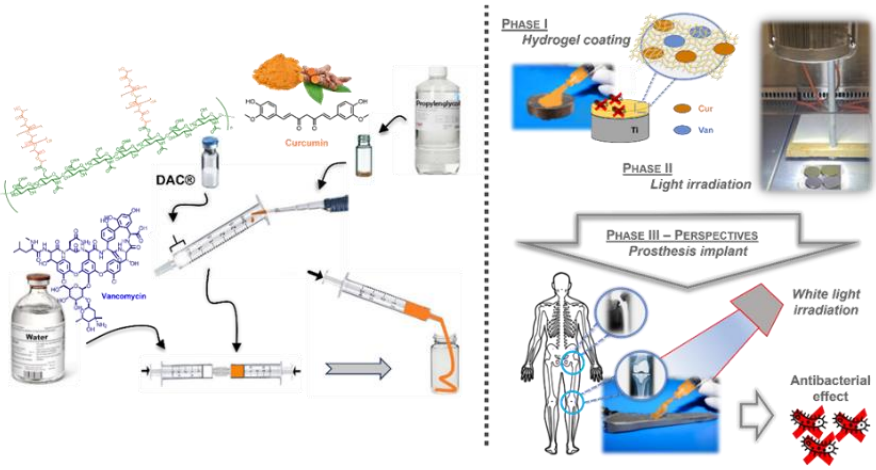

Due to higher life expectancy and to increased expectations of mobility in older age, the number of joint arthroplasties is rising worldwide. Periprosthetic Joint Infection is one of the most important complications of this surgical procedure due to the widespread resistance to antibiotics [1,2]. Therefore, therapeutic strategies and innovative antimicrobial biomaterials are being developed to eradicate pathogens without inducing resistance and accelerating recovery [3]. As part of our ongoing research on supramolecular photosensitizers systems [4], curcumin I (Cur) and vancomycin (Van) loaded DAC®-based (Defensive Antibacterial Coating, a hyaluronic acid and polylactic acid conjugate) hydrogel has been built on. This drug association demonstrated synergistic and additive effects to the single and established vancomycin loading due to the photoinduced broad antibacterial activity of Cur [5] becoming promising for antibacterial photodynamic therapy (aPDT). To incorporate Cur in the hydrogel making it bioavailable in water, a cosolvent method was developed. Hydrogel was prepared and characterized by rheological evaluations and its erosion together with the drug release profile over the time evaluated in biocompatible medium. The nanohydrogel produced upon water dilution was characterized by AFM, DLS technique and UV/Vis absorption and emission spectroscopies. Superior Cur stability over pH-, solvent- and photo-induced degradations resulted in the DAC matrix. The photoinduced antimicrobial activity of Cur/Van-DAC against methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and vancomycin-resistant *Enterococcus faecium* (VRE-fm) was evaluated finding good results against MRSA.

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Lucia Pappagallo

Neurophage: molecular engineering of phage nanoparticles for non-invasive neuronal photostimulation.

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The M13 phage has emerged as a versatile nanocarrier with a wide range of innovative nanobiotechnology applications. Its distinctive filamentous shape, coupled with the arrangement of different coat proteins along its structure, provides an exceptional cargo capacity for genetically fused or chemically conjugated molecules. In addition, the intriguing and unexplored characteristic of M13 phage to cross the Blood-Brain Barrier (BBB), makes it a promising delivery agent for the treatment of different Central Nervous System (CNS) diseases, overcoming challenges in the biomedical field.
The mechanisms that enable M13 to cross the BBB were investigated in vitro on 2D and 3D BBB models. Furthermore, the high cargo capability and ease of genetic handling were exploited to enhance its crossing ability by displaying BBB interacting peptides in fusion with the phage's major coat protein (pVIII).
Additionally, nanobodies were displayed on the phage's minor coat proteins (pIII), to enable the retargeting of the nanobiotechnological platform towards specific cell populations. As proof of concept, an anti-ALFA tag nanobody expressed in fusion with the pIII protein allowed the specific targeting of the phage to engineered neurons expressing a synthetic ALFA-transmembrane protein.
After validation of the BBB crossing ability and targeting specificity, further modifications are currently being introduced to this phage vector platform to target various central nervous system receptors implicated in pathological pathways. These modifications will involve genetic manipulation of mice and chemical conjugation of photovoltaic materials to the phage, with the overarching goal to demonstrate the non-invasive photostimulation of denervated neurons by the Neurophage nanobot.
This platform demonstrates remarkable versatility, potentially serving as an advanced nanocarrier for the delivery of various molecules of interest, such as photosensitizers. Its capability to target and transport these molecules to an anatomically and physiologically challenging to reach region of the human body, such as the CNS, underscores its significant potential in overcoming current limitations in targeted therapy of neurological diseases.

Carlotta Pontremoli

Hafnium based metal–organic framework entrapping squaraines for efficient NIR-responsive photodynamic therapy against pancreatic cells.

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Polymethine dyes (cyanines and squaraines) have emerged as promising candidates [1] as photosensitizer (PS) for photodynamic therapy (PDT), thanks to the possibility to easily tune their structure to have absorption maxima in the near infrared region (650-900 nm), allowing deeper tissue penetration. In addition, their ability to quickly generate ROS at low concentrations is advantageous for reducing potential side effects [2,3]. However, they suffer of low solubility and stability in biological media and nanotechnology has emerged as a promising avenue to address these limitations. Among the different nanosystems, Metal–organic frameworks (MOFs) have metal ions with organic linker molecules that form complex 3D porous structures able to host high amounts of PS [4]. Despite conventional MOFs' limitation to micropores, the development of hierarchically porous MOF (HP-MOFs) has expanded their applicability.

In this work, two asymmetrical squaraines (SQs) bearing bromine and carboxylic moieties, differing in the functionalization on the central core, have been synthesized. After the photochemical characterization, SQs were incorporated by impregnation into different hafnium-based MOFs, designed according to computational analysis of the SQ dimension. SQ-MOFs have been characterized using UV-Vis and Fluorescence spectroscopy, XRD, TGA, N_2 physisorption, and FE-SEM. SQs demonstrated UV-Vis absorption and emission maxima at around 695 nm and 715 nm, matching the therapeutic window. The SQ-MOF systems exhibit similar characteristics to free SQs, suggesting preservation of PS properties. Notably, the incorporation into MOF enhances dye solubility and stability while maintaining ROS generation capability without altering MOF structure. The *in vitro* biological assessments were examined to evaluate their cyto- and phototoxicity against PANC-1 (pancreatic ductal adenocarcinoma cancer cell line). To run a low number of *in vitro* experiments as well as to observe which variables mainly affect the SQ-MOF activity, a statistical multivariate design (Design of Experiments) has been applied by selecting light irradiation time, power and dye concentration as variables, and absorbance, proportional to the cell viability after the irradiation, as response.

This research demonstrates the feasibility of integrating SQ dyes into MOFs, offering a potential solution to their practical limitations and advancing PDT as a therapeutic modality.

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Giovanni Romano

Multicolor endoscopic source for intragastric phototherapy against Helicobacter pylori

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In the context of intragastric phototherapy, different solutions have been developed in time for light delivery, including an inflatable fiberoptic based source, emitting endoscopic blue light[1] and an ingestible luminous capsule provided with LED sources[2]. The endoscopic source is not battery-limited but emits one wavelength range only and does not integrate with existing gastroscopic devices. The capsule is battery-limited but emits multiple wavelengths, expected to optimize the photokilling efficacy according to recent studies[3]. Despite their innovation and the encouraging outcome of clinical trials, none of these solutions have still proved to be effective in achieving infection eradication.

Here, we propose to couple the positive aspects of those devices: the presence of powerful, external light sources and of multiple wavelengths. This defines a new therapeutic device formed by laser sources coupled with an optical fiber, to be further integrated into any gastroscope. This solution takes advantage of current protocols to enter the gastric cavity, allows endoscopic imaging in the pre-, during and post-light delivery phases, and optimizes the operator compliance with the proposed phototherapy approach. The objective of this study is, then, to define and characterize a prototype of such a device.

Following the choice of the optical and mechanical components, laser sources (395, 405, 528, 615 nm)[3] were powered and controlled by dedicated electronics and firmware, driven by a touch pad to set the emission parameters. Safety measures were also defined and implemented in the setup. Laser-fiber coupling was accomplished and optimized by optical components based on Fresnel lenses; tip-diffusing fibers were used to maximize the gastric wall treated area. Light emission was characterized in terms of radiant power and beam geometry. Healthy *ex vivo* stomach models (pig, entire organ) were used to analyze possible photothermal effects on the gastric wall tissue, which were compared with heat diffusion simulation studies and histologic analysis on biopsies. Finally, the correct integration of the prototype with a gastroscope was tested: the two were coupled by inserting the optical fibers in the gastroscope channel.

All the prototype components were correctly working. Integration of up to 3 fibers into a gastroscope was demonstrated. Both fiber and tissue heating were acknowledged, as a function of the wavelength, region, and illumination time; an emission range was defined to avoid tissue thermal damage.

Based on these very encouraging results, further optimization is needed to minimize fiber and tissue heating and acknowledge the device safety in an animal model by defining a suitable treatment plan.

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Simona Salerno

Chitosan-Porphyrin Composite Membrane for Topical Photodynamic Therapy

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The use of photodynamics in dermatology is emerging as new and alternative therapy in anti-cancer treatments, and in many other diseases and defects, as wounds repair and rejuvenation. The challenging applications of these therapies rely in their immunomodulatory, antibacterial, and regenerative properties. At low energy density, photosensitizers enable specific cell/tissue and/or organs response to photostimulation. Polymeric biodegradable membranes as porous barriers provide protection of skin damages ensuring oxygenation and repair. Moreover, as biomimetic microenvironments, membranes represent appealing biomaterials for tissue engineering and regeneration. In this study a biodegradable and microporous composite membrane of chitosan (CHT) and porphyrin was developed as a potential patch for the treatment of skin diseases. CHT membranes have previously been developed and tested as promising biomaterials for dermal and epidermal skin reconstruction [1, 2]. The composite membrane was developed by incorporating in the CHT polymeric solution a photosensitizing nanosystem, based on the cationic porphyrin 5,10,15,20-tetrakis (N-methyl-4-pyridyl)-21H,23H-porphyrin (TMPyP) complexed with the commerical sulfobutyletherbeta-cyclodextrin (CAPTISOL®) [3].
The CHT/CAPTISOL®-TMPyP composite membrane shows a peculiar porous microstructure and evidences a significative antimicrobial activity, opening new insight for skin disease treatments with photodynamic therapy.

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Bianka Siewert

Photoactive Antimicrobials from South American Fungi

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The fruiting bodies of mushrooms contain a diverse array of pigments, many of which have been chemically characterized. However, their biological functions remain largely unknown. Intriguingly, in old chemotaxonomic studies from the 1980s, hypericin was described as a chemical marker for dermocyboid *Cortinarius* species from the Southern Hemisphere (Keller, Moser et al. 1988). At the same time, Gill et al. discovered that this phototoxic pigment is not inherently present in the fruiting bodies but rather formed following insect infestation (Gill, Gimenez and McKenzie 1988). Nevertheless, a systematic investigation trying to explore the ecological function and the distribution of this photobiological phenomenon was just recently initiated by us (Siewert 2021, Siewert, Curak et al. 2022).
To understand the distribution of the photoactivity trait in the subgenus Dermocybe, extensive *Cortinarius* specimen collections were gathered in South America and grouped based on their morphological and phylogenetic features. To annotate known and unknown pigments, we conducted chemical fingerprint analyses and used the DMA-assay to test the extracts for their ability to produce singlet oxygen. Finally, we performed (photo)cytotoxic assays and (photo)antimicrobial tests to explore their potential as new leads in medicinal photochemistry.
Here, we present the promising results, including the selective phototoxic effect of *C. teresae* a mushroom species froming reddish fruiting bodies. Extracts obtained from this species demonstrated an EC_{50} of less than 2.5 µg/mL (478 nm, 9.3 J/cm²) against cells of a bladder cancer cell line (T24), while it showed no effect against *Staphylococcus aureus* and *Candida albicans*.

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Rosalba Sortino

Three-photon infrared stimulation of endogenous neuroreceptors *in vivo*

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To interrogate neural circuits and crack their codes, in vivo brain activity imaging must be combined with spatiotemporally precise stimulation in three dimensions using genetic or pharmacological specificity. This challenge requires deep penetration and focusing as provided by infrared light and multiphoton excitation and has promoted two-photon photopharmacology [1-6] and optogenetics [7-8]. However, three-photon brain stimulation in vivo remains to be demonstrated. We report the regulation of neuronal activity in zebrafish larvae by three-photon excitation of a photoswitchable muscarinic agonist at 50 pM, a billion-fold lower concentration than used for uncaging, and with mid-infrared light of 1560 nm, the longest reported photoswitch wavelength. Robust, physiologically relevant photoresponses allow modulating brain activity in wild-type animals with spatiotemporal and pharmacological precision. Computational calculations predict that azobenzene-based ligands have high three-photon absorption cross-section and can be used directly with pulsed infrared light. The expansion of three-photon pharmacology will deeply impact basic neurobiology and neuromodulation phototherapies.

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Lorenzo Torrisi

Carbon dots luminescence via carbon laser ablation in biocompatible solution.

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Luminescent carbon dots (CDs) were obtained via laser ablation of vegetable carbon targets placed in phosphate-buffered saline (PBS) biological solution.
The carbon particles have a low nanometric size and have been generated by a pulsed IR laser at a 970 nm wavelength, with 100 ms pulse duration, and 350 mJ pulse energy, by using a 10 Hz repetition rate.
ATR-FTIR and UV–Vis spectroscopies, luminescence measurements and optical microscopy were employed to evince the CDs suspension photoluminescence which is induced by a 365 nm UV excitation lamp. The CDs luminescence occurs with a major peak at 478 nm and a second minor peak at 518 nm giving the emitted light a characteristic blue-green color [1].
The quantum efficiency of the process was evaluated.
The CDs luminescence in biocompatible solutions has interesting bioimaging applications in the bio-medical field, such as detection of neoplastic cells or pathogenic bacteria, also including those associated with biofilms. In the last, applications could range from the understanding of interspecies interactions in the development of the biofilms to the detection and typing of specific pathogenic strains, necessary for the choice of the therapeutic approach.
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Mariachiara Trapani

A nanohybrid assembly composed of silver nanoparticles and porphyrins for antimicrobial photodynamic applications

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Multifunctional hybrid nanomaterials composed of organic and inorganic components are extensively studied for the improved performances deriving by the combination of chemically different species, thus providing applications in several fields including biomedical applications [1]. If porphyrins are employed as organic components for the construction of multifunctional nanoscale architectures, their ability to act as photosensitizer able to kill bacteria or virus under light exposure enables the applications in antimicrobial photodynamic therapy (aPDT) [2]. Similarly, the use of silver nanoparticles as antimicrobial agent is extensively known [3]. With the aim to gain a boosting effect from the synergy between the photosensitizer and silver nanoparticles combined in a single nanohybrid materials, this study deals with the synthesis of AgNPs@H_2T_4 nanoassembly composed of meso-tetrakis(N-methylpyridinium-4-yl)porphine (H_2T_4) and silver nanoparticles (AgNPs), finely combined through synthetic procedures based on a supramolecular approach. Metal nanoparticles have been synthesized through reduction metal synthesis assisted by a cyclodextrin based polymer bearing citrate functionalities [4] which acts as stabilizing agent of NPs. The organic covering layer composed of cyclodextrins provides biocompatibility to NPs, which could be transferred into bacteria thanks to the widely proven carrier ability of the organic macrocycle. Several spectroscopic techniques including UV-vis absorption, static and dynamic fluorescence emission, Dynamic Light Scattering and Z- potential measurements, have been used to characterize the nanohybrid. *In-vitro* antibacterial activity has been evaluated against *P. aeruginosa* ATCC 27853 and two clinical isolates characterized by broad-spectrum resistance. Results indicate that AgNPs@H_2T_4 nanoassembly shows the highest biocidal activity when exposed to light, particularly against the two clinical isolates. Our approach allows for a control of the photosensitizer content in core-shell NPs aiming to design novel nanophototherapeutics with promising dual action.

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Sonia Visentin

Assessment of Cystic Fibrosis Mucus Permeability to Fluorescent Bacterial Secretome Molecules for Drug Candidate Potential

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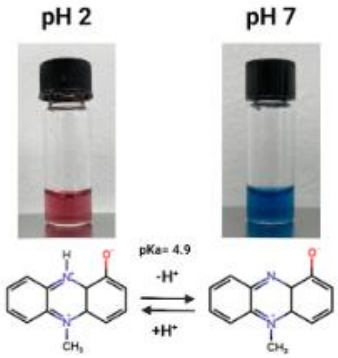
P. aeruginosa employs specific quorum sensing (QS) mechanisms to orchestrate biofilm formation, enhancing resistance to host defences. In physiological conditions, QS molecules permeate the lung environment and cellular membrane to reach the cytoplasmic Aryl Hydrocarbon Receptor (AhR) that is pivotal for activating the immune response against infection [1, 2]. In pathological conditions like cystic fibrosis (CF) this interkingdom communication is altered, favouring P. aeruginosa persistence and chronic infection. Here, we aim to investigate the molecular journey of QS molecules from CF-like environments to the cytoplasm by quantifying via HPLC-MS the permeability through a Permeapad® plate implemented with a mucus model of selected QS molecules (quinolones, lactones, and phenazines) through *in vitro models* of the two main biological lung barriers: CF-mucus and cellular membrane [3].

In particular, we will investigate the behavior of pyocianine, a phenazine compound known for its distinctive blue-green color and redox-active properties. The fluorescence properties of pyocyanine are critical for visualizing its distribution in complex environments such as CF mucus. When modified to include fluorescent markers, pyocyanine becomes a powerful tool for investigating the dynamics of bacterial infections and the efficacy of potential drug candidates.



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Raffaella Margherita Zampieri

Biohydrogen production through photofermentation by a purple non-sulfur bacterium.

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Hydrogen (H_2) is an effective, environmentally friendly, and renewable fuel source that can be produced through biological processes by bacteria and microalgae [1]. Some photoheterotrophic bacteria can convert organic acids into H_2 and carbon dioxide (CO_2) under anaerobic conditions in the presence of light [2]. In particular, purple non-sulfur (PNS) bacteria can use organic compounds (malate, acetate, butyrate, propionate, lactate) as electron donors. This ability is particularly attractive when considering the possibility of using low- or zero-cost waste materials, such as industrial effluents, municipal solid waste, and sewage sludge, while obtaining a clean energy source.

In this study, the PNS bacterium *Rhodopseudomonas palustris* 42OL was cultivated in a 220 mL cylindrical photobioreactor for three weeks under constant illumination (using halogen lamps with a light intensity of 90 W/m^2). Cells were immobilized in calcium-alginate beads with a diameter of 4.1 ± 0.2 mm, corresponding to a total volume of 48.9 mL at a starting concentration of 1.1 g/L of cell dry weight. Cell immobilization has several advantages including cell stability and resistance to mechanical stress, increased cell biomass, and reduced contamination. As a carbon source, 6 g/L of butyrate was supplied in the medium. Several parameters were monitored every hour: pH, oxidation-reduction potential, and H_2 production. For the latter, a calibrated column immersed in a CO_2-absorber solution was used to collect the H_2 produced by the cells. After 7 days of acclimation, the immobilized cells produced 868 mL of H_2 over the next 2 weeks, with an average H_2 production rate of 2.68 mL/h. The consumption of phosphate and sulfate from the medium was also assessed at different time points.

Further research is necessary to improve the industrial and commercial feasibility of H_2 production by photosynthetic microorganisms. Enhancing light conversion efficiency and lowering nutrient supply costs, for instance, could make the process more economically possible.

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Nina Burduja

A Biodegradable Hydrogel based on Captisol®/Porphyrin Nanoassemblies for Antimicrobial Photodynamic Therapy.

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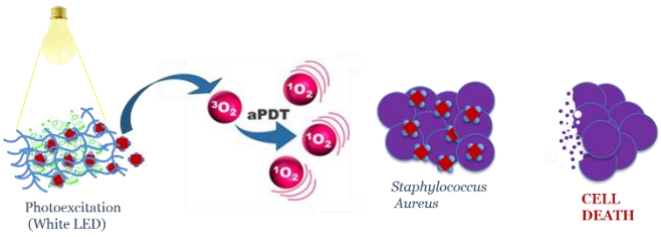
Current research focuses on developing advanced strategies for microbial control in wound treatment, aiming to effectively neutralize pathogens and overcome multidrug resistance (MDR). In the context of our investigation on phototherapy and photodiagnostics, we have designed "smart" nanoassemblies that encapsulate photosensitizers[1,2] (PS) for antimicrobial photodynamic therapy (aPDT). Here, we present the development of rapidly resorbable hydrogels based on DAC® (a copolymer of hyaluronic acid grafted with polylactic acid, HA-PLA) that incorporate a nanophotosensitizer (nanoPS) composed of a cyclodextrin/photosensitizer complex for controlling wound infections. Specifically, the nanocarrier Captisol® (sulfobutyl ether-beta-cyclodextrin) encapsulates the porphyrin 5,10,15,20-Tetrakis-(N-methyl-4-pyridyl)-porphine-Zn(II) tetratosylate (ZnTMPyP), rotecting it from photodegradation and enhancing the efficacy of antimicrobial photodynamic therapy. The CAPTISOL®/ ZnTMPyP complex was prepared by mixing the two precursors in ultrapure water with a molar ratio of CD/PS of 5:1, lyophilized, resuspended in water, and mixed with DAC® powder to obtain DAC® loaded with CAPTISOL®/ ZnTMPyP. To evaluate the efficacy of the hydrogel in local wound infection applications, DAC® loaded with CAPTISOL®/ ZnTMPyP was diluted to 0.3% w/v in DAC®, resulting in a nano-hydrogel characterized as an erosion product at the infection site. The interaction of pure ZnTMPyP and in the presence of cyclodextrin within the hydrogel was investigated using spectroscopic techniques such as UV/Vis absorption, steady-state and time-resolved fluorescence, and Dynamic Light Scattering (DLS). Additionally, the kinetic erosion profiles of DAC®/CAPTISOL®/porphyrin hydrogels were studied in biological media. The nanohydrogels exhibited significant photobactericidal activity against both Gram-positive and Gram-negative bacterial strains, including methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, vancomycin-resistant *Enterococcus faecium* (VREfm) DSM 17050, and VIM-2 producing *Pseudomonas aeruginosa* DSM102273. In vitro photoantimicrobial studies confirmed the aPDT efficacy of our photosensitizing hydrogels incorporating cyclodextrin/porphyrin complexes. These results highlight the potential of DAC® nano-hydrogels loaded with CAPTISOL®/ ZnTMPyP as effective delivery systems for treating wound infections, significantly contributing to the fight against drug-resistant infections.



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Benjamin Clépoint

"Green" Fabrication of Gold Nanostructures for Photothermal therapy.

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The low turnover of new anticancer drugs and the rising antimicrobial resistance call for an urgent shift of attention to unconventional medecines. Photothermal therapy uses controlled heat release, which is localized and can interact with multiple targets, generated under light stimulation for precise treatment control. Gold nanoparticles (AuNPs) are promising for photothermal therapy against cancer and antimicrobial resistance.[1,2]
There is a lack of reports on the green synthesis of anisotropic AuNPs, which are essential for *in vivo* applications.[3] This work aimed to develop green synthetic routes for anisotropic AuNPs using principles of green chemistry. Two nanoscale systems were explored using curcumin and indigo carmine as green reducing agents. A b-cyclodextrin polymer was used as a templating and stabilizing agent and to enhance the solubility of the reducing agents. The characterization of the AuNPs and their photothermal properties was performed. UV-Vis absorbance spectra of both systems showed characteristic bands of anisotropic AuNPs in the visible and near-infrared regions. With an irradiation in the latter, the curcumin-reduced system exhibited a repeatable temperature increase of 14-15°C, while the indigo carmine-reduced system showed a 6°C increase (Fig. 1).

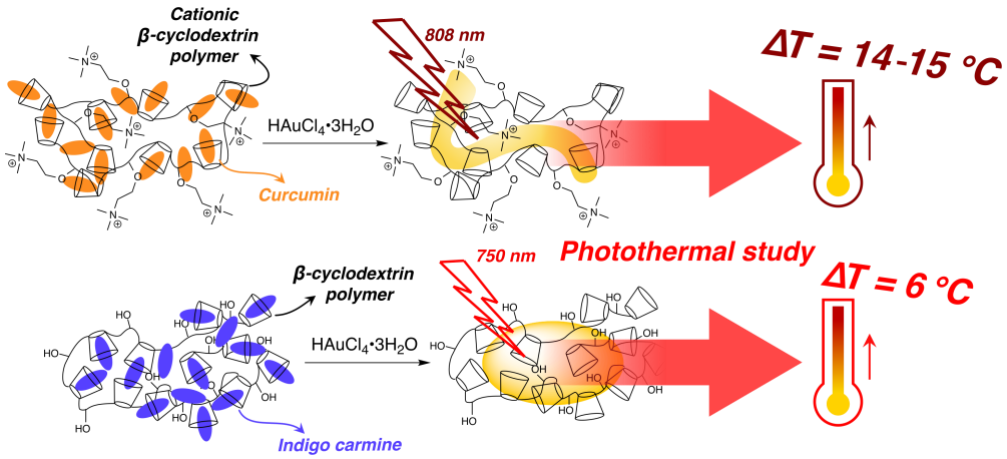

Figure 1: Synthesis of the gold nanoparticle from $HAuCl_4 \bullet 3H_2O$ reduced by curcumin or indigo carmine. Followed by a near-infrared irradiation of AuNPs for heating investigation.

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Elisabetta De Diana

Impact of real-life light-induced stress on Bevacizumab (Avastin®) and Durvalumab (Imfinzi®) during handling in hospital pharmacy: implication on the biological activity.

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Over the last three decades, monoclonal antibodies (mAbs) have undergone a remarkable transformation from scientific tools to powerful human therapies. Due to their unique properties, such as molecular weight, solubility and stability, these biological drugs pose a significant challenge from a pharmaceutical point of view. During their life, mAbs may encounter various situations that can compromise their stability, such as shaking and temperature fluctuation during their transport, and exposure to light during their dilution in intravenous bags and patient's administration, which takes hours. Indeed, stability testing during mAbs manufacturing and developing processes is a critical regulatory requirement, as instability may alter the pharmacological activity of these drugs. However, there are few studies on the stability of these biologics during the real-life (*i.e.* hospital pharmacy handling) and it is of paramount importance to identify the effect of these stressors responsible for mAbs instability.
This study focuses on the effects of real-life light doses on the formulated monoclonal antibodies Bevacizumab (Avastin®) and Durvalumab (Imfinzi®) with or without dilution in saline solution. We found that these mAbs retain their secondary and tertiary conformation, although a decrease in target recognition was observed in *in-vitro* cell assays. We detected the presence of chemical modification (oxidations and deamidations) of some aminoacids by LC-MS/MS analysis, and the formation of aggregates by gel electrophoresis.
In conclusion, a prolonged light exposure of mAbs may result in a reduced pharmacological activity, thus limiting the effectiveness of the clinical treatment.

Acknowledgments
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Miryam Chiara Malacarne

Preclinical in vivo model for application of a BODIPY in aPDT.

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Nowadays, in biomedical research, approximately 20 million animals are used, especially mouse and rat, due to their physiological and immune systems that are nearly equivalent to those of humans [1]. However, high costs and ethical problems are encouraging the scientific community to reduce and replace mammals in research [2]. In this regard, the use of invertebrates presents several advantages including lower costs, ease of manipulation, and although the physiology of insects differs from that of vertebrates, their innate immune system possesses some features functionally comparable to that of vertebrates [3].
Overall, it has been widely demonstrated that invertebrates, particularly nematodes and insects, can be a valid alternative model for preclinical studies on pharmacological molecules and for toxicity studies.
Since the beginning of the 21st century, the insect *Galleria mellonella*, a lepidopteran also known as the Greater Wax Moth (GWM), which in nature is a parasite of beehives, has been used for medical and scientific research purposes [4]. In an increasing number of studies, *G. mellonella* has been validated as an alternative invertebrate model to evaluate the pathogenetic mechanisms of various human pathogens and their interaction with the insect immune system, or to evaluate new therapeutic treatments for infectious diseases [5, 6]. In this work, we evaluated the antimicrobial efficacy of a photosensitizer (PS) on *G. mellonella* [7]. The chosen molecule was 4,4-difluoro2,6-diiodo-1,3,5,7-tetramethyl-8-[2'-(2''-hydroxyethoxy)phenyl)]-4-bora3a,4adiaza-s-indacene, previously synthesized in our laboratories and whose antimicrobial efficacy in vitro on gram positive and negative bacteria has already been evaluated [8].
Firstly, PS's uptake and uptake kinetics in *Micrococcus luteus* bacterial strain was quantified to determine the minimum time necessary for the incubation of PS, and it was observed that after 15 minutes a good degree of uptake was obtained, which remained unchanged over time. Then the minimum bacterial concentration that caused the complete mortality of *G. mellonella* larvae was determined, equal to 10^4 CFU/larva. As the last parameter, the concentration of PS that does not appear to be toxic on the larvae after photodynamic action was evaluated and was found to be equal to 5 µM. Finally, the in vivo photodynamic action was evaluated on larvae infected with *M. luteus* and subsequently treated with PS and irradiated with green LED for 90 minutes. The results highlighted an excellent survival of *G. mellonella* larvae showing the effectiveness of aPDT and the reliability of this insect as for preclinical antimicrobial tests.

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Maura Monforte

Bodipy-Tagged Galactoconjugates and their potential biomedical applications.

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Boron dipyrromethenedifluoride (BODIPY) fluorophores have found widespread application in many branches of biology in the last few decades, due to their optimal properties, as high absorption coefficients in the visible region, good luminescence properties, high photostability, weak dependence on environmental conditions, small size, and finally their chemical versatility for *core* modification with target molecules.[1] The conjugation of fluorophores with sugar moiety can improve the selectivity and solubility in aqueous media. In particular, we have synthesized two luminescent glyconjugated systems (**GalTEBB-1** and **GalTEBB-2**) characterized by two BODIPY moieties and three galactoside rings linked by an oligophenylene ethynylene (OPE) *core*.[2] The bicromophoric system has showed unique photophysical behavior, working as artificial antenna systems with extremely efficient and fast coulombic energy transfer [3], that could be useful in fluorescence imaging.
In this poster communication we will describe the synthetic pathway that exploits a series of copper-free Heck–Cassar–Sonogashira cross-couplings and the early bactericidal tests of the two luminescent glycoconjugates showed in figure below.

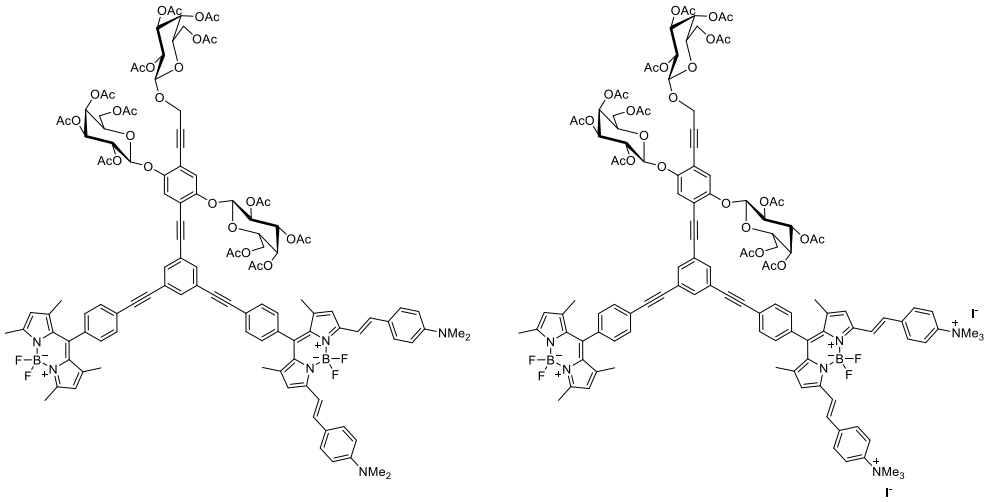

Figure. **GalTEBB-1** and **GalTEBB-2**

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Faye Naessens & Elena Catanzaro

Development of near-infrared photosensitizers for deep-tissue cancer treatment.

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Photodynamic therapy (PDT) is a clinically approved, minimally invasive form of anticancer treatment. It involves the administration of drugs, called photosensitizers (PSs), which are inert but acquire cytotoxic potential when irradiated at a wavelength specific for each compound. The light promotes PSs activation, which transfers energy to molecular oxygen to produce singlet oxygens that rapidly react with cellular components, causing oxidative damage and ultimately leading to the death of tumor cells. In this regard, an immunogenic form of cell death, called immunogenic cell death (ICD), can be induced, which not only eliminates the tumor cells, but also activates an anti-tumor immune response and establishes enduring immunological memory. Commonly used PSs for the treatment of tumors, such as melanoma, are excited by wavelengths ranging from 600 to 700 nm. However, these wavelengths cannot penetrate deep into tissues (only a few mm) and are only used to treat easily accessible tumors. As near-infrared (NIR) light offers a deeper tissue penetration depth (10-40 mm), the use of PSs excited in the NIR spectrum overcomes this limit and allows PSs activation in large and deep-tissue tumors. Moreover, the use of lipid-based nanoparticles, which are one of the most successful nano-delivery vehicles, can improve the delivery of the PSs and induce an enhanced and tumor-specific PDT effect. Hence, the objective of this project is to evaluate the anticancer and immunogenic properties of sensitive NIR dyes (provided by Agfa-Gevaert NV), whether or not encapsulated in lipid-based nanoparticles, on *in vitro* cancer models. In this study, we performed a screening of 60 NIR-PS molecules and identified three molecules that are able to promote oxidative stress and induce immunogenic cell death.