Italian Society of Photobiology XXXIII Annual Conference

Akademie Cusanus – Bressanone 18-19 July 2022

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Conference Program

July 18 - 19, 2022 Annual Conference Program

Monday 18 July 2022

8:30 - 9:00	REGISTRATION
9:00 - 9:15	**OPENINGS Valentina Rapozzi - Giorgia Miolo**
Chair	**Francesco Milano**
9:15 - 9:45	**Emiliano Altamura** "Semi-synthetic bottom-up approach for photosynthetic artificial cell construction"
9:45 - 10:00	**Paola Albanese** "The implementation of chloroplasts as oxygen-producing organelles in artificial cell"
10:00 - 10:15	**Rossella Labarile** "Biocompatibility of dopamine with the photosynthetic growth of R. sphaeroides"
10:15 - 10:30	**Lilian Torquato** "Intact photosynthetic bacteria-based biophotoanodes exposed to nitrophenol contaminants"
10:30 - 11:00	**COFFEE BREAK**
Chairs	**Massimo Trotta - Giorgia Miolo**
11:00 - 11:30	**Kristjan Plaetzer** "Adding Photodynamic Inactivation to the farmer's toolbox to fight plant pathogens"
11:30 - 11:45	**Michael Glueck** "Sodium magnesium chlorophyllin based sunlight-PDI against plant pathogenic Clavibacter michiganensis"
11:45 - 12:00	**Christoph Hamminger** "Photodynamic Inactivation of fungal plant pathogens"
12:00 - 12:15	**Andreas Fellner** "Fresh, safe and healthy food: Photodynamic Decontamination of seeds and sprouts from Listeria innocua"
12:15 - 12:30	**Viviana Orlandi** "Light at 410 nm controls the growth of contaminant bacteria in fishery field"
12:30 - 14:00	**LUNCH BREAK**
Chairs	**Valentina Rapozzi - Enrico Caruso**
14:00 - 14:10	**YOUNG INVESTIGATORS AWARD**
14:10 - 14:35	**Greta Avancini** "Keratin nanoparticles and photodynamic therapy enhance the anticancer stem cells activity of salinomycin"
14:35 - 15:00	**Matteo Di Giosia** "Bio-Conjugated Fullerenes: Innovative Platforms for Phototheranostic Applications"
15:00 - 15:25	**Luca Menilli** "Light-Activated Nitric Oxide Generators for Cancer Therapy"
15:25 - 16:00	**COFFEE BREAK**
Chairs	**Carlo Musio - Matteo Calvaresi**
16:00- 16:30	**Benoit Piro** "Electrolyte-gated transistors for monitoring photosynthetic organisms"
16:30 - 16:45	**Greta Varchi** "Prodrugs-based nanoparticles combining anticancer chemo- and photo-therapy"
16:45 - 17:00	**Sonja Visentin** "Switch on the mucin, a powerful cancer biomarker"
17:00 - 17:15	**Rosa Matteucci** "Bio-based electrodes in photobioelectrochemical systems for environmental monitoring"
17:15- 17:30	**Nina Burduja** "Sulfobutylether βeta cyclodextrin / porphyrins nanocomplexes for pathogens sensing"
17:30-17.45	**Carlo Musio** "Light regulation of transcripts of classic and unconventional opsins in the eyeless cnidarian Hydra"
17:45 - 19:00	**SIFB meeting (shareholders only)**
20:30	**SOCIAL DINNER**

Tuesday 19 July 2022

Chair	**Antonino Mazzaglia - Greta Varchi**
8:45 - 9:15	**Fabienne Dumoulin** "Updates on the use and role of phthalocyanines in PDT"
9:15 - 9:30	**Miryam Malacarne** "Synthesis and evaluation of the biological activity of porphyrin-peptide conjugateswith specific targeting against TNBC "
9:30 - 9:45	**Marzia Gariboldi** "Free and poly-methyl methacrylate-bounded BODIPYs for photodynamic therapy"
9:45: 10:00	**Benedetta Fongaro** "Photostability studies on therapeutic monoclonal antibodies: the case of Ipilimumab"
10:00 - 10:15	**Nadia Barbero** "Quatsomes loaded with a squaraine dye as powerful nanovesicles for Photodynamic Therapy"
10:15 - 10:30	**Matteo Calvaresi** "Engineered bacteriophages: Effective viral vectors for receptor targeted anticancer photodynamic/sonodynamic therapy"
10:30 - 11:05	COFFEE BREAK
Chair	**Francesca Moret - Viviana Orlandi**
11:05 - 11:20	**Carlo Matera** "Photoswitchable molecular prosthetics to photocontrol auditory neurons"
11:20 - 11:35	**Mariachiara Gani** "The Role of Nitric Oxide in the bystander effect in photooxidized prostate cancer cells"
11:35 - 11:50	**Giacomo Insero** "First Prototype of an Innovative 222 nm Device for the Suppression of Airbone Viral Epidemic Spread"
11:50 - 12:05	**Francesco Garzella** "Innovative Ultraviolet light barriers for the Suppression of Airborne Viral Epidemic Spread"
12:05 - 12:20	**Annette Wimmer** "Tackling antimicrobial resistance in agriculture: Photodynamic Inactivation of Erwinia amylovora"
12:20 - 12:35	**Giovanni Romano** "The role of biofilm optical properties in the modulation of photokilling efficacy: a theoretical model"
12:35 - 14:00	**LUNCH BREAK**
Chair	**Marina Venturini - Giovanni Romano**
14:00 - 14:30	**Franz Trautinger** "Phototherapy for cutaneous T cell lymphomas"
14:30 - 14:45	**Mariateresa Rossi** "Deeds and misdeeds about sunscreens"
14:45 - 15:00	**Carlotta Pontremoli** "Antimicrobial PDT: nanoparticles incorporating cyanines and squaraines as new nanophotosensitizers"
15:00 - 15:15	**CLOSING CERIMONY**

Conference keywords
Applied Photobiology and Biophysics
Optical and spectroscopic methods applied to biology, medicine and biosafety
Light -responsive materials
In vitro photodynamic investigations: focus on antimicrobial and antitumoral strategies
Light and human health

Book of abstracts

Invited speakers

Emiliano Altamura

Semi-synthetic bottom-up approach for photosynthetic artificial cell construction.

Altamura Emiliano[1*], Paola Albanese[2], Francesco Milano[3], Massimo Trotta[4], Pasquale Stano[5] and Fabio Mavelli[1*]
[1] Chemistry Department, Università degli Studi di Bari Aldo Moro,Via E. Orabona 4, 70125, Bari, Italy.
[2] Department of Physical, Natural and Environmental Sciences (DSFTA), University of Siena, Italy.
[3] CNR-ISPA, Institute of Sciences of Food Production, Lecce Unit, Lecce, Italy.
[4] CNR-IPCF, Institute for Physical and Chemical Processes, Bari, Italy.
[5] Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Lecce, Italy.

A continuous energy supply is a critical requirement in the implementation of synthetic cells from scratch that can be considered alive or, at least, maintained in homeostatic conditions far from equilibrium. In this contribution, two different approaches preparation of photosynthetic artificial cells, that are phospholipid giant unilamellar vesicles (GUVs) capable of transducing light energy into chemical energy, will be presented and discussed [1]. the first foresees that every single enzyme involved in the bacterial photosynthetic process is extracted and reconstituted in the GUV membrane with the right physiological orientation [2,3]; the second pursues the extraction of the entire photosynthetic apparatus in form of organelles, nanometric bacterial vesicle called chromatophores, capable of carrying out the phosphorylation of ADP in ATP under continuous light irradiation when trapped in the aqueous lumen of GUVs [4]. To prove that in these artificial cells a metabolic pathway can be sustained by light energy, a transcription kit has been encapsulated inducing the synthesis of RNA molecules fuelled by the ATP photo-produced. The transcription process is the first step of protein expression, that is a key process in the life cycle of living organisms.

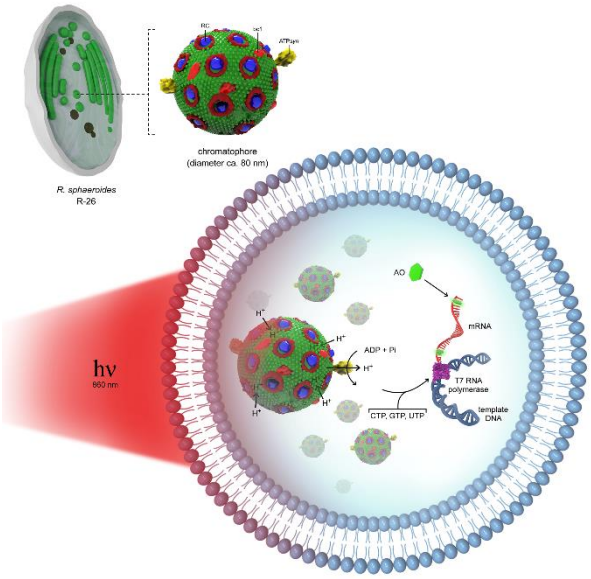

Figure 1. Schematic drawing of a photosymthetic artificial cell entrapping chromatophores, the light energy transducing bacterial compartments.[4]

[1] Altamura E., et al. (2021). The Rise of the Nested Multicompartment Model in Synthetic Cell Research, *Frontiers in Molecular Biosciences 8*, 750576 Authors (Year).
[2] Altamura E. et al. (2017). Highly oriented photosynthetic reaction centres generate a proton gradient in synthetic protocells. *PNAS 114*(15), 3837-3842.
[3] Altamura E. et al., Optimizing Enzymatic Photo-Redox Cycles by a Hybrid Protein Complex Chain. *Chem. Photo Chem. 5*(1), (2021), 26-31.
[4] Altamura E., et al., Chromatophores efficiently promote light-driven ATP synthesis and DNA transcription inside hybrid multi-compartment artificial cells, *PNAS 118*(7), (2021) e2012170118

Fabienne Dumoulin

Updates on the use and role of phthalocyanines in PDT

Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Medical Engineering Department, Ataşehir, Istanbul, Turkey

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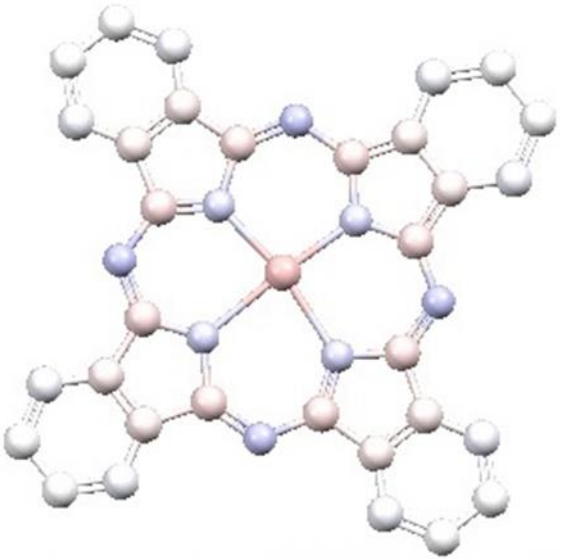
Photodynamic therapy is now more than promising but some drawbacks still must be overcome. Tissue light penetration and protection of endogenous chromophores are some of them. With their maximum absorption around 700 nm, phthalocyanines are recognized as amongst the best photosensitisng cores.[1] Our group is developing several strategies and research directions to improve their efficiency, by improving their photoproperties, the tumor targeting, combining the photodynamic effect with other therapeutic and/or imaging effects.



Our latests results will be presented in the context of current state of the art.

[1]Pui-Chi Lo, M. Salomé Rodriguez-Morgade, Dennis K. P. Ng, Tomas Torres, Ravindra Pandey and Fabienne Dumoulin, *Chemical Society Reviews*, **2020**, *49*, 1041-1056

Kristjan Plaetzer

Adding Photodynamic Inactivation to the farmer's toolbox to fight plant pathogens.

Annette Wimmer[1], Andreas Fellner[1], Michael Glueck[1], Christoph Hamminger[1], Jun Liu[2], Michael Fefer[2], Kristjan Plaetzer[1]

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Plant diseases caused by bacterial or fungal pathogens lead to severe harvest losses. Pathogens that enter the food supply chain, for example by spoilage of fruits or vegetables by bacteria present in manure, can induce severe outbreaks of foodborn disease. Photodynamic Inactivation represents an effective tool to kill microorganisms and, if based on natural photosensitizers combined with sunlight activation, might allow for economic and ecofriendly application to fight plant pathogens and increase food safety and shelf life. We here prove that sodium magnesium chlorophyllin (Chl) combined with cell wall permeabilizers, or B17-0024, a cationic derivative of chlorin e6, are effective in killing relevant bacterial and fungal plant pathogens such as *Clavibacter michiganensis* (causing bacterial wilt and canker), *Erwinia amylovora* (fire blight, [1,2]), *Xanthomonas axonopodis* (citrus canker, [1]) and *Botrytis cinerea* (grey mold, [3]). Hundred micromolar of the photoactive compound and 30-120 minutes of sunlight activation are sufficient to induce a reduction of viable bacteria by seven orders of magnitude (7 log steps, 99.99999%). Fungal growth of *B. cinerea* is abolished *in vitro* after PDI using 100 µM of B17-0024 and LED-illumination at 395 nm (106.6 J/cm^2). As demonstrated by a plant compatibility assay using *Fragaria vesca* neither the photosensizers, nor the additives influence growth and development of the host plants [3]. Sodium magnesium chlorophyllin (food additive E140), curcumin (E100, bound to polyvinylpyrrolidone, E1201) or its cationic derivative SACUR-3 are applicable for post-harvest Photodynamic Decontamination of seeds, sprouts and vegetables. Depending on the geometrical properties of the foodstuff, the bacterial load *in situ* can be reduced by 3 log steps on slices of peppers (flat object, PVP-curcumin, *Staphylococcus aureus*, [4]) and 4 log steps on mung bean seeds (round object, Chl, *Listeria innocua*) to 6 log steps on salad (flat object, SACUR-3, *Escherichia coli*, [5]). As conclusion, PDI is an effective treatment of plant pathogens and able to improve microbial food safety.

[1] Glueck, M., Hamminger, C., Fefer, M., Liu, J., and Plaetzer, K. (2019). Save the crop: Photodynamic Inactivation of plant pathogens I: bacteria. *Photochemical & Photobiological Sciences*, *18*(7), 1700-1708.

[2] Wimmer, A., Glueck, M., Ckurshumova, W., Liu, J., Fefer, M., and Plaetzer, K. (2022). Breaking the rebellion: Photodynamic Inactivation against Erwinia amylovora resistant to streptomycin. *Antibiotics*, *11*, 554.

[3] Hamminger, C., Glueck, M., Fefer, M., Ckurshumova, W., Liu, J., Tenhaken, R., and Plaetzer, K. (2022). Photodynamic Inactivation of plant pathogens part II: fungi. *Photochemical & Photobiological Sciences*, *21*(2), 195-207.

[4] Tortik, N., Spaeth, A., and Plaetzer, K. (2014). Photodynamic decontamination of foodstuff from Staphylococcus aureus based on novel formulations of curcumin. *Photochemical & Photobiological Sciences*, *13*(10), 1402-1409.

5] Glueck, M., Schamberger, B., Eckl, P., and Plaetzer, K. (2017). New horizons in microbiological food safety: Photodynamic Decontamination based on a curcumin derivative. *Photochemical & Photobiological Sciences*, *16*(12), 1784-1791.

Benoit Piro

Electrolyte-Gated Transistors for Monitoring Photosynthetic Organisms.

Benoit Piro, Jérémy Le Gall, Nicolas Battaglini, Sandra Vasilijevic, Giorgio Mattana, Vincent Noël

Laboratoire ITODYS, Université Paris Cité, 75006 PARIS.

Among organic thin film transistors (OTFTs), Electrolyte-Gated Organic Field-Effect Transistors (EGOFETs) have scarsely been described for living cell monitoring. However, EGOFETs are well adapted for this application because they can operate directly in aqueous solutions such as cells culture media and offer high on/off ratio which leads to high sensitivity. To start, I will explain or re-explain the operation mechanisms of EGOFETS. Then, as a proof of concept, I will propose to monitor the photosynthetic activity of a cyanobacterium (Anabaena flos-aquae) contained within an EGOFET's electrolyte. In this case, photosynthesis is continuously monitored by electroreduction of oxygen produced or consumed by the cyanobacteria, on the gate electrode. As an extension, I will show that the microorganisms can be entrapped and kept alive in an hydrogel servig as gate in an EGOFET (HGOFET), an architecture that can be used for the detection of water pollutants, as an example, and for environmental monitoring in general. Some other examples will be taken from the literature, and I will explain that printing processes can used for making such devices.

[1] Le Gall, J., Vasilijević, S., Battaglini, N., Mattana, G., Noël, V., Brayner, R., Piro, B. *Electrochimica Acta* 372, 137881
[2] Le Gall, J., Mouillard, F., Le, T.N., Vu, T.T., Mattana, G., Brayner, R., Zrig, S., Noël, V., Piro, B. *Biosensors and Bioelectronics* 157, 112166

Franz Trautinger

Phototherapy for Cutaneous T-cell Lymphomas.

[1]Department of Dermatology and Venereology, University Hospital of St. Poelten, Karl Landsteiner University of Health Sciences and [2]Karl Landsteiner Institut of Dermatological Research; St. Poelten, Austria

Cutaneous T-cell lymphomas (CTCL) are a heterogenous group of non-Hodgkin lymphomas presenting primarily in the skin. Mycosis fungoides (MF) is its most common variant characterized by a chronic course of slowly progressing cutaneous patches and plaques. In some patient further progression to tumours and extracutaneous involvement occurs, associated with a poor prognosis. Particularly in early disease stages remission can be induced in most patients with narrow-band ultraviolet B radiation (NB-UVB) or ultraviolet A radiation combined with 8-methoxypsoralen (PUVA). Sézary syndrome (SS), a rare and aggressive variant of CTCL, is characterised with generalized skin and blood involvement. Extracorporeal photochemotherapy (ECP), where peripheral blood is exposed to PUVA, has been successfully used alone or in combination in these patients. Lymphomatoid papulosis (LP) is a CD30+ lymphoproliferative disease, characterised by chronically recurring papules. LP responds favourably to PUVA, but low dose methotrexate might be preferred for long term disease control.

All available treatment guidelines agree in the importance of phototherapy for disease control in CTCL. Areas of uncertainty are the lack of standardized treatment protocols, the indication for maintenance therapy, the value of ultraviolet A1, excimer sources, and photodynamic therapy and the potential contribution of UV-induced mutagenesis to the course of the disease.

Young Investigator Award

Greta Avancini

Keratin nanoparticles and photodynamic therapy enhance the anticancer stem cells activity of salinomycin

Greta Avancini[1], Andrea Guerrini[2], Claudia Ferroni[2], Daniele Tedesco[2], Marco Ballestri[2], Marta Columbaro[3], Luca Menilli[1], Elena Reddi[1], Roberto Costa[1], Luigi Leanza[1], Greta Varchi[2], Francesca Moret[1].

[1]Department of Biology, University of Padova, Padova, Italy; [2]Institute of Organic Synthesis and Photoreactivity, Italian National Research Council, Bologna, Italy; [3]IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy.

Breast cancer (BC) is the most common malignant tumours in women with an estimated 2.3 million new cases in 2020 [1]. Increasing evidence shown that breast cancer stem cells (BCSCs), which possess self-renewal and tumour-initiating capacity, are promoting therapy resistance, recurrence, and metastasis. Therefore, the development of therapeutic strategies for the selective targeting and killing of BCSCs besides differentiated cancer cells, very likely in combination with other treatments, as for example photodynamic therapy (PDT), could be a successful strategy. Among repurposed drugs that were screened as potentially effective toward cancer stem cells eradication, salinomycin (SAL) is the most potent since it is able to induce ROS formation and apoptosis/ferroptosis in a wide panel of cancer types [2]. Moreover, SAL can inhibit Wnt/β-catenin signalling and target the Hedgehog pathway, thus preventing the maintenance of the CSCs pool. Nonetheless, clinical administration of SAL is limited due to its poor water solubility and the consequent unfavourable pharmacokinetic [3]. To solve this issue and propose a combined therapeutic protocol for BC we synthetized wool keratin nanoparticles (SAL/Ce6@kVEs) for the delivery of SAL in combination with chlorin e6 (Ce6), a PDT photosensitizer (PS), and vitamin E acetate.

SAL/Ce6@kVEs were prepared in water by nanoprecipitation, using exclusively VE acetate as stabilizing agent; they showed an average hydrodynamic diameter of 127 nm and maintain the capacity to efficiently produce singlet oxygen upon light irradiation. *In vitro* cytotoxicity studies performed in two different BC cell lines (MDA-MB-231 and MCF-7), highlighted the occurrence of synergism between SAL chemotherapy and Ce6-based PDT as well as confirmed the capacity of SAL/Ce6@kVEs of reducing stemness potential and inhibit the formation of CSC-enriched mammospheres. *In vivo* studies on zebrafish embryos confirmed the ability of SAL delivered by NPs to inhibit the Wnt/β-catenin signalling pathway, which is dysregulated in BC, thus identifying a target for further pre-clinical investigations.

[1] Harbeck, N., Penault-Llorca, F., Cortes, J. *et al* (2019). Breast cancer. *Nat Rev Dis Primers.* **5**, 66. [2] Mai, T. T. *et al* (2017). Salinomycin kill cancer stem cells by sequestering iron in lysosomes. *Nat Chem.* **9**, 1025-1033. [3] Ojo, O. O. *et al* (2013). Dose-dependent adverse effects of salinomycin on male reproductive organs and fertility in mice. *PLoS.* **8**, e69086.

Matteo Di Giosia

Bio-Conjugated Fullerenes: Innovative Platforms for Phototheranostic Applications

Matteo Di Giosia[1], Alice Soldà[2], Markus Seeger[2], Andrea Cantelli[1], Fabio Arnesano[3], Maria I. Nardella[3], Vincenzo Mangini[3], Francesco Valle[4], Marco Montalti[1], Francesco Zerbetto[1], Stefania Rapino[1], Vasilis Ntziachristos,[2] Matteo Calvaresi[1]

[1]NanoBio Interface Lab, Dipartimento di Chimica "Giacomo Ciamician", Alma Mater Studiorum—Università di Bologna, via Francesco Selmi 2, Bologna 40126, Italy; [2] Chair of Biological Imaging Center for Translational Cancer Research (TranslaTUM) Technical University of Munich, 81675, Munich, Germany; [3]Dipartimento di Chimica, Università di Bari "A. Moro", via E. Orabona 4, Bari 70125, Italy, [4] Istituto per lo Studio dei Materiali Nanostrutturati (CNR-ISMN), Consiglio Nazionale delle Ricerchevia P. Gobetti 101, Bologna, 40129, Italy

Fullerenes are candidates for theranostic applications because of their high photodynamic activity and intrinsic multimodal imaging contrast. However, fullerenes suffer from low solubility and tendency to aggregate in aqueous media, poor biocompatibility and cell toxicity.

Noncovalent bioconjugation of fullerenes with proteins is an emerging approach for their dispersion in water. Contrary to covalent functionalization, the supramolecular approach preserves the physicochemical properties of the carbon nanostructures. The unique photophysical and photochemical properties of fullerenes are then fully accessible for applications in different fields, from materials science to nanomedicine.

The hybridization of fullerenes with proteins allows the production of innovative multifunctional theranostic platforms where the role of proteins is akin to that of "Trojan Horses" since they can i) hide the fullerene from the biological *milieu*, ii) control their cellular uptake, iii) govern their biological fate.[1]

C_{70}@lysozyme is introduced herein as a novel photoactive bioconjugate and optoacoustic contrast agent for tracking cellular uptake and intracellular trafficking.[2]

The excellent imaging contrast of C_{70}@lysozyme in optoacoustic and third harmonic generation microscopy was exploited to monitor its uptake in HeLa cells and its lysosomal localization. The photodynamic activity of C_{70}@lysozyme caused cell death by means of singlet oxygen ($^1O_2$) production upon exposure to low intensity white light irradiation. This study introduces fullerene-protein conjugates as potential candidates for phototheranostic applications.

[1] Di Giosia, M.; Zerbetto, F.; Calvaresi, M. Incorporation of Molecular Nanoparticles Inside Proteins: The Trojan Horse Approach in Theranostics. (2021) *Accounts Mater. Res. 2*(8), 594–605.

[2] Di Giosia, M.; Soldà, A.; Seeger, M.; Cantelli, A.; Arnesano, F.; Nardella, M. I.; Mangini, V.; Valle, F.; Montalti, M.; Zerbetto, F.; Rapino, S.; Calvaresi, M.; Ntziachristos, V. A Bio-Conjugated Fullerene as a Subcellular-Targeted and Multifaceted Phototheranostic Agent. (2021). *Adv. Funct. Mater. 31*(20), 1–8.

Luca Menilli

Light-Activated Nitric Oxide Generators for Cancer Therapy

Menilli L.[1], Parisi C.[2], Failla M.[2], Fraix A.[2], Moret F.[1], Reddi E.[1], Rolando B.[3], Spyrakis F.[3], Lazzarato L.[3], Fruttero R.[3], Gasco A.[3], Sortino S.[1]

[1] Department of Biology, University of Padova, Italy
[2] PhotoChemLab, Department of Drug and Health Sciences, University of Catania, Italy
[3] Department of Science and Drug Technology, University of Torino, Italy

The use of reactive oxygen species (ROS) and reactive nitrogen species (RNS) generators as "unconventional" therapeutics with precise spatiotemporal control by using light stimuli may open entirely new horizons in innovative therapeutic modalities for cancer. Among ROS and RNS, nitric oxide (NO) plays a dominant role for its potent oxidizing power and cytotoxic action. Besides its cytotoxic effect, nitric oxide also has the potential to nitrosylate proteins, particularly those involved in the onset of multi-drug resistance. These molecular hybrids therefore have the potential to be exploited for combination therapy. In our work, two different molecular hybrids, called BPT-NO and DXNO-GR, were synthesized by conjugating a light-harvesting antenna moiety to an N-nitroso appendage, which can trigger the release of NO following light irradiation with specific wavelengths. The biological activity of the two molecular hybrids was evaluated on different human cancer cell lines, and they were well tolerated in the dark, while cell viability was dramatically affected when irradiated. The red fluorescence emission of these compounds was exploited to quantify their cellular uptake and to detect their intracellular localization. The ability of nitric oxide generated by molecular hybrids to inhibit the activity of the MDR1 efflux pump was also verified, thus confirming that this class of compounds have the potential to open new horizons in cancer therapy.

Oral Communications

Paola Albanese

The implementation of chloroplasts as oxygen-producing organelles in artificial cell

Paola Albanese[1], Emiliano Altamura[2], Divesh K. Baxani[3], Yuval Elani[3], Fabio Mavelli[2]

[1] Department of Physical, Environmental and Natural Sciences, University of Siena, 53100 Siena, Italy.
[2] Chemistry Department University of Bari Aldo Moro, 70125 Bari, Italy.
[3] Chemical Engineering Department, Imperial College London, W12 7TA London, United Kingdom.

The construction of artificial cells from scratch is one of the most challenging goals in bottom-up Synthetic Biology and is mainly pursued by implementing complex cell-mimicking behaviours in simplified compartments. Generally, the building blocks used to assemble these micrometric constructions are a combination of simple-synthetic and bio-extracted molecules (e.g., proteins, nucleic acids, or even entire organelles) following a semi-synthetic bottom-up strategy.

In this framework, our research group has been working to implement the photosynthetic process in artificial cells thus awarding the inanimate compartment with the capability of autonomously generate chemical energy from light. In previous years, this result was achieved by enclosing in the aqueous core of Giant Unilamellar Vesicles (GUVs) bacteria-extracted chromatophores, acting as photosynthetic organelle-like modules [1]. Nowadays, as a follow-up of the chromatophore-based multicompartment system, we are investigating chloroplasts and thylakoid membranes as further modular building blocks in the construction of photo-active artificial cells. Chloroplasts are way more complex than chromatophores and could perform multiple tasks for a synthetic cell under a light trigger i.e., producing oxygen, synthetising high-energy molecules (ATP and NADPH) and fixing CO_2. Here, these organelles are proposed, both in bulk and encapsulated, as oxygen photo-producers capable of improving O_2 availability in the local microenvironment (Figure 1). Indeed, O_2-evolving biomaterials are of main interest in biomedical applications for oxygen delivery to hypoxic tissues in the absence of a vascular supply [2]. As proof of concept, we tested if oxygen evolution by isolated organelles, in an anoxic atmosphere, could generate favourable conditions for the oxygen-dependent maturation process of the Green Fluorescent Protein chromophore as model reaction to occur.

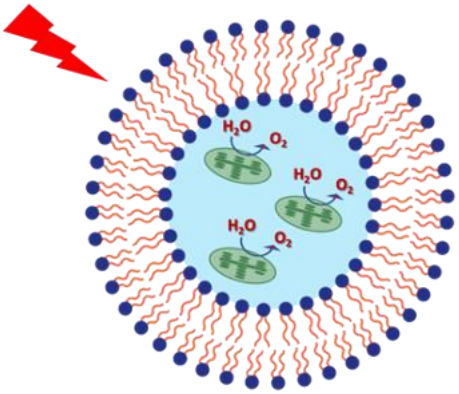

Figure 2. Schematic representation of a chloroplast-containing artificial cell evolving molecular oxygen under actinic illumination.

References

[1] Altamura, E., et al. (2021) Chromatophores Efficiently Promote Light-Driven ATP Synthesis and DNA Transcription Inside Hybrid Multi Compartment Artificial Cells. Proc. Natl. Acad. Sci. USA 118 (7):e2012170118.
[2] Chen, P.S. et al., (2020) Pathophysiological implications of hypoxia in human diseases. Journal of Biomedical Science 27, 63.

Rossella Labarile

Biocompatibility of dopamine with the photosynthetic growth of R. sphaeroides

Rossella Labarile[1,2], Danilo Vona[2], Gabriella Buscemi[1,2], Maria Varsalona[1,2], Matteo Grattieri[1,2], Gianluca M. Farinola[2], Massimo Trotta[1]

[1] CNR-IPCF; [2]Università degli Studi di Bari A. Moro.

Living and metabolically active microorganisms can be used in biohybrid devices for the sustainable production of energy [1]. The purple non-sulfur bacterium *Rhodobacter (R.) sphaeroides* is a photosynthetic prokaryote able to convert sunlight in other forms of energy by photosynthesis that can be exploited in such biohybrid devices.

Intimate contact between the bacterial cell membrane and the electron acceptor surface is required. The optimization of the interfaces of poorly conductive bacteria with electronic components can be achieved using biocompatible polymers that encapsulate and stick the bacterial cells over the electrode [2]. Polydopamine a bioinspired polymer produced by the self-polymerization of dopamine, can be used as bacterial coating due to its excellent adhesivity and low toxicity while its redox-active functional ligands can be exploited for bioelectric applications [3, 4]. Biocompatibility of dopamine with the photosynthetic growth of *R. sphaeroides* was tested after additions of the monomer to the culture media.

Spectrophotometric characterizations and Transmission electron microscopy (TEM) analysis of ultra-thin sections of bacterial cultures exposed to dopamine were performed at increasing concentration of the monomer.

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Lilian D. M. Torquato

Intact photosynthetic bacteria-based biophotoanodes exposed to nitrophenol contaminants

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The deep understanding of the light energy transduction process, as well as the exploitation of the outstanding metabolic versatility of purple non-sulphur bacteria, are of great interest for the application of photobioelectrochemical systems. In these biohybrid devices, sunlight is used to produce bioenergy and target chemical compounds as well as for the decontamination and monitoring of water environments. The development of efficient biosensing and biophotovoltaics platforms, however, requires the creation of a coherent electrochemical interface between the biophotocatalyst and the electrode surface to harvest the photoinduced electron produced. Recent reports elucidated the mechanisms involved in the electron transfer process as well as showed the most recent groundbreaking achievements toward the practical application of intact and viable photosynthetic microorganisms in biohybrid electrochemical systems.[1] Our group recently developed an innovative and sustainable one-pot approach to obtain a redox-adhesive polydopamine matrix that simultaneously facilitates the photoinduced extracellular electron transfer while maintaining the bacteria in close contact with the electrode surface.[2] Here we demonstrate the possibility to utilize these biohybrid photoelectrodes for the on-line, early monitoring of contaminants in water environments by studying the effects of different concentrations of 2,4 dinitrophenol on photobioelectrocatalysis of purple bacteria. Electrochemical evidence of nitrophenols on *Rhodobacter capsulatus* photosynthetic chain will be presented, discussing the possibility to utilize the developed systems as sustainable biosensors for environmental monitoring.

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Michael Glueck

Sodium magnesium chlorophyllin based sunlight-PDI against plant pathogenic Clavibacter michiganensis

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Clavibacter michiganensis is a Gram+ bacterial plant pathogen that causes bacterial wilt and canker of tomato, which results in economic losses by killing young plants or by reducing the yield. It is seedborne and has spread around the globe [1]. Photodynamic Inactivation (PDI) based on sodium magnesium chlorophyllin (Chl) has been shown to be effective against bacterial and fungal plant pathogens under laboratory conditions [2-4]. The aim of this study was to compare the efficiency of Chl-based PDI against *C. michiganensis* using artificial LED-light and natural sunlight. The bacterial suspension was incubated with 1, 10 and 100 µM Chl for 5 or 30 min in the dark followed by illumination using a LED-array (395 nm wavelength, 26 J/cm² radiant exposure). For the transition from the laboratory to the real situation in the field, the bacteria were mixed with 100 µM Chl in liquid culture and placed in direct sunlight for illumination. The irradiance was logged and the radiant exposure was calculated in real-time. At 12.5 J/cm², 25 J/cm² and 50 J/cm² samples were taken and evaluated. PDI of *C. michiganensis* using 26 J/cm² LED-light resulted in a relative inactivation ($CFU_{control}/CFU_{sample}$) of 6.7 log_{10} steps at 100 µM concentration of Chl (5 min incubation). The relative inactivation at 12.5 J/cm², which was acquired after 25-30 min illumination with sunlight at an Austrian autumn day, reached 6.5 log_{10} and increased to 8.1 log_{10} at 25 J/cm² and to 7.4 log_{10} at 50 J/cm². In this study we proof, that effective PDI of *C. michiganensis* can be achieved by illumination with artificial light or sunlight. In addition to activation of the Soret band by the LED array, natural sunlight is able to excite further peaks in the absorption spectrum of Chl. Therefore, comparable photoantibacterial effects are induced at about half of the radiant exposure. Given the high photokilling in short illumination periods (30 min) sunlight-PDI is well useable in agriculture.

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Christoph Hamminger

Photodynamic Inactivation of fungal plant pathogens.

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The ever-increasing demand for agricultural products and the resulting monocultures, as well as the excessive use of fungicides, lead to fungi that are resistant to conventional treatments. This calls for new methods to control fungal outbreaks. The two fungal species employed in this study - *Botrytis cinerea* as well as *Alternaria solani* cause considerable economic losses and crop failures. Sodium magnesium chlorophyllin (Chl, approved as E140) combined with Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) and B17-0024 (B17-0024 Ce6-15,17-DMAE, a chlorin e6 derivative carrying cationic moieties) are used as photosensitisers (PS). Photodynamic Inactivation (PDI) efficiency was evaluated by mycelial sphere growth inhibition (average diameter 2–3 mm) after incubation with the PS for 100 min and illumination using a LED array (395 nm, 106.6 J/cm²). One hundred micromolar Chl with 5 mM Na_2EDTA successfully photokilled 94.1% of *A. solani* and 91.7% of *B. cinerea* samples. B17-0024 completely inactivated *A. solani* at 10 times lower concentration (10 µM) but for *B. cinerea*, the concentration required for complete eradication was (100 µM). A *Fragaria vesca* PDI-plant compatibility assay was used to demonstrate that both PS do not cause significant leaf damage or negatively affect host plant development. [1]

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Andreas Fellner

Fresh, safe and healthy food: Photodynamic Decontamination of seeds and sprouts from *Listeria innocua*.

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Home growing of sprouts by consumers provides fresh and healthy food. However, precautions have to be taken, as bacterial contaminations on seeds might multiply during germination in a humid and warm atmosphere, resulting in a concerningly high microbial load of sprouts. Photodynamic Decontamination (PDc) helps to increase food safety by killing of bacteria via light-induced and photosensitiser mediated overproduction of reactive oxygen species [1]. This study investigates the application of PDc based on sodium magnesium chlorophyllin (Chl) for microbial control of Listeria innocua inoculated on mungbean (Vigna radiata), radish (Raphanus sativus var. sativus) and buckwheat seeds (Fagopyrum esculentum). Microbial load of the seeds after PDc, their germination rate and the resulting contamination of sprouts are determined. Incubation of mungbeans with 100 µM Chl for 30 min and subsequent illumination (LED array 395 nm, 56.5 J/cm^2) reduces the bacterial load of seeds by more than 99.99%. Both, the dry weight and germination rate of seeds are not influenced by PDc when compared to untreated controls. Decontamination of seeds transfers to germinated sprouts, resulting in a 99.9% reduction of Listeria innocua after storage for 10 days in the fridge. Photoinactivation based on Chl is also effective on radish seeds (99.9% reduction at 28.2 J/cm^2) and less than 90% reduction at 28.2 J/cm^2 in buckwheat. As conclusion, PDc allows for reduction of bacteria relevant for food safety on seeds and sprouts without impact on the germination rate. This procedure might help to avoid foodborne disease caused by Listeria and increase shelf life of sprouts.

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Viviana Orlandi

Light at 410 nm controls the growth of contaminant bacteria in fishery field

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Antimicrobial Blue Light (aBL) approach is a light-based technique that exploits the antimicrobial activity of light emitting in the blue range. It has been hypothesized that specific wavelengths can activate potential endogenous photosensitizers in microbial cytoplasm and/or envelope. The arisen photooxidative stress compromises macromolecules and induces inactivation of microorganisms. This approach can be used to control the growth of pathogens and contaminants in several applicative fields, from sanitization of inert surfaces to human skin treatments, from industry to food and feed fields.

In particular, in fishery world, microbial contaminants represent a reservoir of potential pathogens dangerous for human health and responsible for degradation of flesh. Nowadays, icing and freezing are the most used techniques to control fish spoilage. Furthermore, UV treatment and chemical disinfections are also considered. These treatments often worsen the taste and smell of fresh fish, compromising the quality of food, aBL opens new perspectives.

In this study, we focused the attention on a demersal teleost fish *Chelidonichthys lucerna* as model fish to irradiate with LED at 410 nm. The approach was efficient in greatly reducing the microbial community colonizing the skin of the fish. The bacterial photoinactivation was light-dose dependent and strain-dependent. Indeed, microorganisms isolated from *C. lucerna*, belonging to different Gram-negative (*Aeromonas* sp., *Exiguobacterium undae*, *Acinetobacter* sp., *Acinetobacter baumannii*) and Gram-positive species (*Bacillus thuringiensis*, *Listeria* sp.) showed a different sensitivity to blue light. This preliminary study highlights that blue irradiation could slow down the spoilage of fish with food quality maintenance, help preserving human health and bring, in addition, economic advantage [1].

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Greta Varchi

Prodrugs-based nanoparticles combining anticancer chemo- and photo-therapy.

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The tumor environment features, such as the EPR effect, elevated levels of glutathione and reactive oxygen species [1], can be conveniently exploited for designing selective and responsive carriers capable of improving the therapeutic outcome. To this purpose, the in situ covalent binding of drugs or nanoparticles to circulating human serum albumin (HSA) represents a promising approach to achieve an effective strategy. Human serum albumin (HSA) is the most abundant blood plasma protein, with an average half-life of three weeks and a hydrodynamic radius of around 7 nm, that has shown great potential for drug delivery thanks to its physiological role as a carrier protein for endogenous and exogenous substances [2].

This study describes the synthesis, in vitro and in vivo characterization of bioresponsive HSA-binding nanoparticles (MAL-PTX2S@Pba), co-delivering two different paclitaxel (PTX) prodrugs and the photosensitizer pheophorbide a (Pba), for the combined photo- and chemo-treatment of breast cancer [3].

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Sonja Visentin

Switch on the mucin, a powerful cancer biomarker

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Up to now different methods to detect mucins have been developed: antibody-based enzyme-linked immunosorbent assays and aptamer-based electrochemical and fluorescence techniques [1] are the most frequently described. Even though commonly adopted, these methods often have limitations regarding relative instability, complex production, difficult purification processes, and are time-consuming. The research for better alternatives is still ongoing. Among the most promising detection methods, fluorimetric assays have received remarkable attention due to their convenience, unparalleled sensitivity, simplicity, rapid implementation, non-invasive monitoring capability and usability in biological samples. To take advantage of this technique in the biomedical field, the interest in developing new dyes that can non-covalently bind specific proteins for their detection is constantly rising up. Particularly, fluorescent probes with absorption and emission in the near-infrared (NIR) region (650–900 nm) are useful for practical biological applications as NIR signal detection does not suffer from self-absorption and autofluorescence typical of biological matrices. Particularly, the fluorogenic and chromogenic probes, which turn-on their fluorescence in response to a biological target, find application for living processes, medical diagnosis and biological imaging at the molecular, cellular and organism level. Of utmost interest in biomedicine is the development of selective fluorescent probes to detect disease-associated biomarkers in biological fluids. Here we are exploiting the fluorescence turn-on behavior manifested by different fluorescent probes as a response of the binding to proteins [2]. The goal is to find and develop a turn-on fluorescent probe which is specific and selective for mucins at serum level. Dye-protein interaction was studied by UV-Vis and fluorescence spectroscopies in aqueous media. Since albumin is the most abundant protein at serum level, it was used as control to compare the turn-on recorded in the presence of mucin. Generally, dyes showed low fluorescence intensity and quantum yield in water, as a result of the formation of aggregates. However, a significative enhancement in fluorescence was achieved when mucin was added. Since mucins can be explored as a diagnostic and prognostic biomarker in cancer disease, these results pave the way for the development of fluorescent dyes as potential biosensors for mucin glycoproteins detection.

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Rosa Maria Matteucci

Bio-based electrodes in photobioelectrochemical systems for environmental monitoring

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Photobioelectrochemical systems are emerging as a key enabling technology for a more sustainable transition in a variety of applications such as chemical synthesis, environmental sensing and remediation, and energy conversion and storage.[1,2] The intrinsic value proposition of their sustainability is based on the use of living micro-organisms as photoelectroactive components in place of the critical raw materials (CRMs) usually adopted in current artificial systems. In this perspective, it is of paramount importance to develop bio-based strategies and solutions also for the abiotic component of such systems, i.e., the electrodes. Carbon based electrodes are often the best choice, but their application on a large scale can be challenging, depending on the specific material used, and the specific electrode shape and size.[3]

In this context, we developed a simple route to obtain a bio-based composite flexible electrode combining a bio-polymer matrix with electrically conductive carbon nanofibers (CNFs). We selected poly-hydroxyalkanoates (PHAs), a family of bacterial thermoplastic polyesters, as the best candidate thanks to the combination of their superior bio-degradability and their excellent thermo-mechanical properties. Following our recent findings,[4] we developed a bio-inspired hybrid photo-anode based on photosynthetic purple bacteria encapsulated onto PHA-CNFs electrodes. For the bacteria encapsulation onto the electrode surface, we used a semiconductor biopolymer, i.e., polydopamine (PDA), which, thanks to its cathecol and amine moieties facilitates the adhesion of intact bacteria while preserving their metabolism and facilitating the electrons transfer. Here we present the use of this bio-based and bio-inspired hybrid system for photocurrent generation and photo-induced monitoring of emerging aqueous contaminants.

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Carlo Musio

Light regulation of transcripts of classic and unconventional opsins in the eyeless cnidarian *Hydra*

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Opsins play a key role in the ability to sense light both in image-forming processes and in non-visual photoreception or circadian vision (i.e., photoentrainment: the entrainment of a cellular oscillator by the light/dark cycle) [1]. Regardless of these two modalities, in the animal phyla the photoreceptor protein in the great majority of cases is an opsin-based protein binding to a light reactive chromophore by a Lys residue (base of Schiff) [2]. So far, GPCRs containing Lys have been discovered neither in sponges nor in porifera while new classes of opsins have been identified in the photoresponsive *Hydra*, an eyeless cnidarian considered the evolutionary sister species to bilaterians [3-5]. Aimed to verify whether light influences and modulates the gene expression of known opsins in *Hydra*, we utilized four opsin EST sequences, belonging to two classic opsins and two non-visual opsins, to investigate, by means of a quantitative RT-PCR, the expression patterns during both photic *Zeitgeber* (from the German for "time-giver", an entrainment signal) and circadian time. In particular, we utilized the mRNA sequences expressed by the following opsin genes: Melanopsin-like, GenBank: DT617488; SW Rhodopsin-like, GenBank: CN554795; SW Blue sensitive opsin-like, GenBank: CN775258 and Peropsin-like, GenBank: CB073527.1. The expression levels followed the light hours of diurnal cycle with respect to the darkness ones and in constant dark condition the relative expression increased. The monophasic behaviour in L12:D12 cycle turned into a triphasic expression profile during the continuous darkness condition. Consequently, while the diurnal opsin-like expression reveals a close dependence on light hours, the highest transcript levels in D12:D12 leaving us to hypothesize an "internal" biological clock which autonomously provided the opsins expression during the circadian time. Therefore, the irradiance, acting as the main temporal indicator of the day-time, would downregulate the circadian mechanism to better optimize the opsins expression to the varying demands of night and day. In conclusion, in *Hydra* the irradiance would regulate the diurnal expression of visual and non-visual opsins as already demonstrated in higher invertebrate and vertebrate species. Our data confirm that the eyeless cnidarian *Hydra* represents a suitable model for studying ancestral precursor of both visual and non-visual photosensitive modalities providing useful hints on the evolution of visual and photosensory systems [6].

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Nina Burduja

Sulfobutylether βeta cyclodextrin / Porphirins nanocomplexes for pathogens sensing

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The detection and monitoring of pathogen bacteria in water resources is a current challenging issue. Within our ongoing research on photodiagnostics, here we propose complexes based on the trade sulfobutylether-beta-cyclodextrin (Captisol®) entrapping the cationic porphyrin (N-methyl-4-pyridyl)-21H,23H-porphyrin (H_2T_4) [1, 2] as nanomaterials for pathogen sensing. Nanoassemblies were prepared at different Captisol®/porphyrin molar ratio, at high entrapment efficiency by simple mixing components aqueous solutions and characterized by UV-Vis and Steady –State Fluorescence Emission, Dynamic Light Scattering and ζ-potential. In order to fabricate a diagnostic kit for pathogen sensing, stability of the nanocomplexes was studied in ultrapure and simulated sea water, in different containers and temperatures mimicking selected sea basins. In order to evaluate the sensing properties of free H_2T_4 and Captisol®/H_2T_4 nanoassemblies, the interaction with a wild strain of E. coli isolated from wastewater was investigated by steady-state and time-resolved fluorescence emission. Spectroscopic results preliminarily pointed out on cationic porphyrin binding with bacterial cell, both as free fluorophore and delivered by cyclodextrin. These evidence open the way to the development of novel nanodiagnostics based on cyclodextrin and porphyrins.

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Miryam Chiara Malacarne

Synthesis and evaluation of the biological activity of porphyrin-peptide conjugates with specific targeting against Triple Negative Breast Cancer (TNBC).

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Breast cancer is the second most common cause of cancer death, and its incidence is increasing worldwide[1]. TNBC, an aggressive variant of breast cancer, is characterized by lack of expression of the estrogen (ER) and progesterone receptors (PRs) and the overexpression of human epidermal growth factor receptor (EGFR) [2]. Treatment options are limited so TNBC is an important area of research for both researchers and clinicians. Recently it has been demonstrated that peptides sequences binding EGFR are efficiently internalised by TNBC cells. The association of photosensitisers (PSs) with peptides has been extensively exploited to improve the performance of porphyrin-based anticancer PSs agents[3].

This project aims at generating porphyrin-peptide conjugates targeting TNBC cells via a novel approach relying on the aromatic displacement of a suitable leaving group on the porphyrin with a thiol group present on the peptide.

5,15-diarylporphyrin were synthetized via acid-catalysed mixed condensation of dipyrromethane with aromatic aldehyde; porphyrins thus obtained was subsequently mono nitrated in meso position. Different peptide sequences, that binds to EGFR, are used as a cancer cell targeting peptide. All the sequences are provided with one cysteine residue at the N-terminus to obtain the desired conjugate via aromatic nucleophilic substitution of nitro groups. From the chemical-physical analyses, conjugates seem to be good candidates for PDT.

The conjugates were tested on TNBC cell line MDA-MB231 and MDA-MB453[4]. The MTT assay performed to evaluate cell survival following treatment for 24 h with the compounds and 2 h irradiation with white light (irradiance of 22 mW/cm^2 equal to 158 J/cm^2 of fluence) showed that all compounds are endowed with photodynamic activity. EGFR overexpression in MDA-MB231 also results in a good uptake of the conjugates in this cell line. From data obtained with flow cytometric analyzes, following the photodynamic treatment MDA-MB231 cells prefer necrosis instead of apoptosis; MDA-MB453 cells undergo apoptosis instead of necrosis. Autophagy, detected by evaluating LC3 levels, would seem not to be particularly induced in MDA-MB231 cells following PDT; on the contrary MDA-MB453 cells increase LC3 levels. The compounds were also evaluated for their ability to inhibit the migration phenomenon that is typical of this highly metastatic cancer such as TNBC. None of the compounds showed antimigration abilities on the cell line.

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Marzia Bruna Gariboldi

Free and poly-methyl methacrylate-bounded BODIPYs for photodynamic therapy.

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Photodynamic therapy (PDT) has emerged as a potential, minimally invasive therapeutic regimen in which a photosensitizer (PS), a light absorbing-molecule, oxygen and a light source with a suitable wavelength enable the production of reactive oxygen species (ROS) inducing cancer cell death. Despite PDT in vitro and in vivo applications are growing, its use in clinical oncology is still limited, mainly due to some relevant limitations, such as phototoxic and photoallergic adverse effects, PSs suboptimal tissue penetration, complex synthetic pathways and difficult chemical modifications to modulate their photophysical and biological properties. In addition, as for chemotherapy, the design of efficient drug delivery systems represents a major challenge in PDT. Concerning this last issue, in recent years, various nanoparticles (NPs) have been developed as drug carriers. Hence, the synthesis and characterization of new PSs or new nanoparticles are actively pursued by many research groups, including ours, both to overcome the limitations described above and to obtain a more efficient drug-delivery. Over the last decade, a new class of photosensitizers based on the 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene core, commonly known as BODIPYs, has attracted considerable attention.

In this work, two new positively charged BODIPY derivatives (**3** and **6**) with different substituents at the meso position of the di-pyrrolyl-methenic nucleus have been electrostatically loaded onto negatively charged poly-methyl methacrylate nanoparticles. The effect of the free and PMMA-bounded compounds on cell viability, along with their cellular uptake and their ability to induce ROS and $^1O_2$, apoptosis, necrosis and/or autophagy and to inhibit spontaneous cell migration were evaluated on a small panel of 2D- and 3D-cultured human cancer cell lines. Our results indicate that PMMA nanoparticles can improve the photodynamic effects of **3** and **6**. In particular, PMMA-bounded BODIPYs displayed a higher internalization degree in tumour cells, both as monolayer and 3D spheroids, and exerted significantly improved anti-migration activity, compared to the free compounds.

Benedetta Fongaro

Photostability studies on therapeutic monoclonal antibodies: the case of Ipilimumab

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The photostability of the monoclonal antibody Ipilimumab, the active ingredient of Yervoy® for the treatment of different types of cancer, has been investigated. Two different doses of artificial solar light were applied to observe the physicochemical and biological behaviour of the molecule under this stressor. The irradiation of 720 kJ/m^2 (200 W•hours/m^2 in the UV region (320-400 nm) corresponded to the minimum exposure requirement of the ICH Q1B guidelines. On the other hand, the provided amount of simulated solar radiation of 10460 kJ/m^2 was used to maximize the light stressor, following the exposure criteria for "stress test" photostability studies. Far and near–UV circular dichroism (CD), dynamic light scattering (DLS), size-exclusion chromatography (SEC), native and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) were performed to evaluate biophysical stability. Finger printing by trypsin was used to check the chemical modifications. When stability conditions were compromised, the viability of human cell lines treated with the stressed formulation has been applied as an indicator of potential biological toxicity. Protein oxidation, deamidation, and aggregation occurred when exposing both the original and saline or glucose diluted preparations to light radiations to which Ipilimumab could be submitted during its real-life. Therefore, physicochemical properties, integrity, and stability studies could assure the best storage and manipulation conditions for the safe and successful application of Ipilimumab in cancer therapy.

Nadia Barbero

Quatsomes loaded with a squaraine dye as powerful nanovesicles for Photodynamic Therapy

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Organic Near Infrared (NIR) polymethine dyes (i.e Squaraines, SQs and Cyanines, CYs) have attracted considerable attention for a wide range of application such as PDT and theragnostic, thanks to their excellent photochemical properties with strong absorbance in the NIR, perfectly matching the phototherapeutic window [1]. Nevertheless, their poor solubility and stability in physiological conditions still limit their wide application. To overcome these drawbacks, a possible solution would be the incorporation of these dyes inside nanoparticles (NPs) to prevent the formation of dye aggregates in aqueous media and protect their photochemical characteristics.

Quatsomes (QS) are thermodynamically stable vesicular structures, constituted by quaternary ammonium surfactants and sterols. These colloidal structures are stable for periods as long as several years; their morphologies do not change upon rising temperature or dilution and show outstanding vesicle to vesicle homogeneity regarding size, lamellarity, and membrane supramolecular organization [2].

In the present work we employed Quatsomes as nanocarriers for two squaraine dyes (Br-C4 and Br-C12) with good photodynamic activity but poor solubility in physiological conditions. QS composed of Cholesterol (Chol) and Sterealkonium Chloride (STK) were loaded with different concentrations of these two SQs. These formulations have been proved to have a high dye loading efficiency even at the higher tested concentrations. This high incorporation efficiency can enable the use of lower amounts of nanovesicles since they can carry higher PSs concentrations, lowering the carriers' cytotoxicity due to the presence of quaternary ammonium surfactants. The formulations under study have been characterized and interrogated *in vitro* for the cytotoxicity and PDT efficiency.

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Matteo Calvaresi

Engineered bacteriophages: Effective viral vectors for receptor targeted anticancer photodynamic/sonodynamic therapy

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Bacteriophages, or simply phages, are widespread viruses that affect bacteria but are inactive against eukaryotic cells. Phages can undergo an extremely flexible genetic engineering allowing for a plethora of targeting design and thus can be easily transformed in effective delivery systems.

We developed an orthogonal nanoarchitectonics approach (genetic/chemical) to engineer M13 bacteriophages as targeted vectors for efficient photodynamic/sonodynamic killing of cancer cells. M13 was genetically refactored to display on the phage tip a peptide [1,2] or a nanobody able to bind the epidermal growth factor receptor (EGFR). Using an orthogonal approach to the genetic display, $M13_{EGFR}$ phages were then chemically modified, conjugating hundreds of photo/sono sensitizers on the capsid surface ($M13_{EGFR}$-PS) [1,2].

The efficient retargeting of phage to cancer cells was qualitatively and quantitatively proved through flow cytometry and confocal microscopy experiments on cancer cell lines overexpressing EGFR [1,2]. The killing activity of cancer cells is observed at picomolar concentrations of the phage vector in both PDT/SDT [1-3]. The $M13_{EGFR}$-PS bioconjugates also present a high permeation into 3D spheroids, proving an increased capability of the M13 phage platform to penetrate 3D cultures in comparison to other targeting agents.

The developed orthogonal (genetic/chemical) strategy for engineering M13 bacteriophages for receptor targeted (EGFR-targeted) anticancer PDT/SDT reveals that: (i) the phage tropism may be easily varied to target different receptors overexpressed in cancer cells; (ii) theranostic platforms may be developed conjugating both therapeutic and imaging tags on the phage capsid.

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Carlo Matera

Photoswitchable molecular prosthetics to photocontrol auditory neurons

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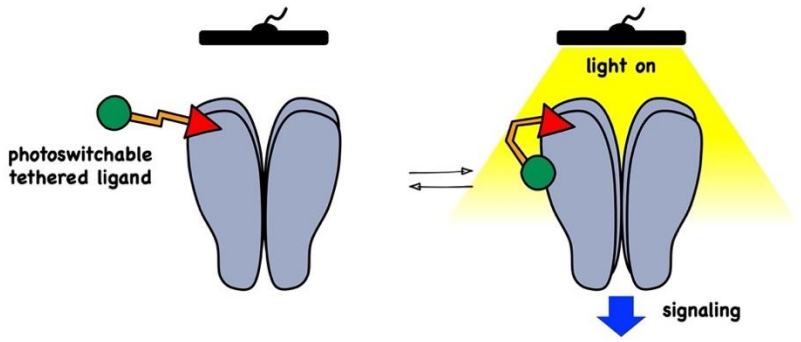
Light-controllable chemical tools provide powerful means to manipulate and interrogate biological functions with high spatiotemporal precision and low invasiveness. Our research efforts in the field have focused on the design of reversible photoswitchable compounds to photocontrol enzymes, GPCRs, and ion channels. We have developed phototrexate, the first photoswitchable inhibitor of the human dihydrofolate reductase with demonstrated cytotoxicity in vitro and in zebrafish larvae [1], PAI, a light-controlled dualsteric agonist of muscarinic M_2 receptors that enabled the photomodulation of cardiac function in tadpoles and of brain states in mice [2,3], and azodopa, a photoswitchable dopamine D_1 receptor agonist that was used to photocontrol swimming behavior in zebrafish larvae and neural activity in mouse cortex [4]. More recently, we have designed a fast photoswitchable tethered ligand of ionotropic glutamate receptors to enable control of the auditory neurons. This compound, named TCP_{fast}, induced photocurrents in untransfected neurons upon covalently tethering to endogenous glutamate receptors and activating them reversibly with visible light pulses of few milliseconds. We applied it to the ultrafast synapses of cochlear auditory neurons that encode sound and provide auditory input to the brain. TCP_{fast} functions as a molecular prosthesis that bypasses the neurotransmitter-encoded signal with a photonic signal. Photosensitization of cochlear spiral ganglion neurons (SGNs) by locally administered TCP_{fast} enabled temporally precise light-evoked SGN firing up to a rate of approximately 1 kHz, matching the fastest optogenetic SGN stimulation. Hence, TCP_{fast}-mediated photopharmacology might serve as an interesting alternative to the optogenetic approach for the development of an optical cochlear implant for hearing restoration [5]. The results of these studies will be presented and discussed.



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Mariachiara Gani

The Role of Nitric Oxide in the bystander effect in photooxidized prostate cancer cells.

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Photodynamic therapy (PDT) is a therapeutic modality used in cancer treatment. The therapy consists of the simultaneous action of three elements: a photosensitizer, a light source and molecular oxygen, which are capable of causing oxidative damage that blocks tumor growth by generating singlet oxygen and reactive oxygen species (ROS). PDT is also responsible for stimulating inducible nitric oxide synthase (iNOS), the enzyme that generates nitric oxide (NO). Our studies have shown that prostate cancer cells effectively upregulate iNOS and consequently stimulate the release of NO when exposed to PDT treatment.

Previous our results reported that the role of NO in PDT treatment depends on the intensity of the insult. Low doses of NO may promote the development of a more aggressive tumor population, while high doses may halt tumor growth and stimulate apoptosis [1].

There is increasing evidence that ROS/RNS can control the cells surrounding PDT-treated tumor cells (so-called bystander cells) [2-3]. Since NO is a gaseous signaling molecule, we postulated that it might act as a "messenger" that tells bystander cells what their fate is.

To address this question, different kind of prostate cancer cells were treated with a mild photooxidative stress, and, 2 hours after irradiation, the supernatant of prostate cancer cells treated with Pb*a*/PDT was transferred to untreated prostate tumor cells mimicking the neighboring "bystander" cells. Our results suggest that low NO levels induced by suboptimal PDT treatment may stimulate proliferation of bystander prostate cancer cells depending on the malignancy grade of the tumor cells.

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Giacomo Insero

First Prototype of an Innovative 222 nm Device for the Suppression of Airbone Viral Epidemic Spread.

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At the beginning of the Covid-19 epidemic, when no vaccines were available, people entrust their safety to very few devices such as personal protective equipment (face masks, shields, and gloves) and social measures such as lock-down and physical distancing. Unfortunately, viruses can persist in the air for a long time after their ejection from the patient, via coughing or sneezing, in the form of droplets and droplet nuclei [1]. From this perspective, the epidemic exposed the lack of alternative and not conventional techniques to suppress the spread of airborne epidemics based on the reduction of virus concentration in the air. The classical approach is based on air exchange, but this is not always a viable or efficient way (for example in a very crowded place or when access to non-contaminated air is not straightforward). The SAVES-US project (Suppression of Airborne Viral Epidemic Spread by UV-Light Barriers) aims at developing and demonstrating an innovative antimicrobial device based on a 222 nm-illuminator. We know that UVC radiation (200-280 nm) is the most effective wavelength for the inactivation of viruses and bacteria, but may also be mutagenic. For this reason, UVC-light sterilization is commonly performed in the absence of living organisms. A small range in the far-UVC region, which includes 222 nm, shows a good antimicrobial efficacy, tested already on both bacteria [2] and virus [3] models, but very limited risks to human health. Thanks to a few µm 222-nm penetration depth, the far-UVC radiation is absorbed by the very superficial stratum corneum of the skin that contains dead cells, thus preventing light absorption for the underlying live tissue [4].

After a brief description of the SAVES-US project, we will show the first prototype of our 222 nm-illuminator together with some preliminary results on its characterization; the presented device will be used in successive in vitro and in vivo experiments with SARS-CoV-2 virus. The device embeds a far-UVC lamp emitting at 222 nm, optical filters, and the controlling electronics. We show results on the spatial homogeneity of the emission intensity and the dependence on the lamp-virus distance. We also report on the ozone production due to absorption of far-UVC light from molecular oxygen naturally present in the air in order to evaluate its safety for human beings and to properly evaluate its photo-killing efficacy.

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Francesco Garzella

Innovative Ultraviolet light barriers for the Suppression of Airborne Viral Epidemic Spread

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Despite the gradual return to pre-pandemic conditions, the spreading of COVID-19 (SARS-CoV-2) left several open issues. Nowadays it is know that airborne infections, including COVID-19, are conveyed by particles having the size of >5 µm (droplets) and <5 µm (droplets nuclei), ejected by coughing and sneezing [1]. While droplets undergo to dehydration and precipitation, droplet nuclei persist in air for long time after their ejection, contributing to infection spreading. Actual prevention strategies are based on non-pharmaceutical interventions act to reduce droplets diffusion and spacing from Personal Protective Equipment, such as facial masks, and social distancing measure. Nevertheless, for the new endemic phase of COVID-19 the development of new strategies for airborne infections' containment becomes unavoidable.

In this project, we designed a brand-new device generating Ultraviolet Light Barriers for the suppression of Airborne Viral Aerosols (SAVES-US) designed to work in situations with constrained geometries (e.g. public transportation, offices, waiting rooms etc.) not allowing social distancing. The device, devised to perform photokilling of viral aerosols in air in presence of humans is based on an UV illumination system operating at 222 nm. It is know from literature that UV radiation alters the genetic material of viruses and bacteria whose maximum absorption wavelengths are in the far-UV range (UVC, 100-280 nm), the most effective for sterilization [2]. Differently from the operative wavelength of most commercial systems (254 nm), the higher tissue absorption prevents the 222 nm radiation to travel over the very first epidermal layers [3] resulting in reduced health risk for applications in presence of people. The device combines the UV illumination system with a vertical flux of air that conveys exhaled particles to the light source and controls humidity and temperature, crucial parameters for virus diffusion.

After its development, the device prototype will be tested in model experiments. Initially, its safety will be proved by monitoring in particular the UVC-induced ozone production. Then, *in vitro* photokilling experiments will be performed in two steps: (i) on a layer of immobilized SARS-Cov-2 virus act to obtain optimal UV doses for an effective sterilization; (ii) on SARS-Cov-2 aerosol models. For this last experiment, we will take advantage of preliminary studies on model viral aerosol miming the characteristics of cough and sneeze particles, supported by synthetic data, to characterize the optical properties of the reference scenario. The resulting information will be crucial for the final design of the device itself. As a last step, we will test the device in *in vivo* experiments. An air flux, harvesting exhaled air by infected mice, will be illuminated by the device and will be sent to healthy mice. Finally, the infectiveness of exhaled air after the UV treatment will be evaluated, providing more information for further applications in the presence of humans.

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Annette Wimmer

Tackling antimicrobial resistance in agriculture: Photodynamic Inactivation of *Erwinia amylovora*.

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Application of streptomycin still serves as golden standard for treatment of fire blight in many countries. As a consequence, *Erwinia amylovora*, the causative bacterial pathogen of this plant disease, has evolved resistance leading to a ban on this antibiotic in Europe. Therefore, very limited strategies remain for farmers to protect their crops from fire blight. Photodynamic Inactivation (PDI) represents an innovative method for killing microorganisms and has been proven effective against plant pathogens. This study aims at investigating whether PDI is applicable for controlling *E. amylovora* susceptible (*E. amylovora* ^{WT}) as well as resistant (*E. amylovora* ^{SmR}) to streptomycin. As eco-friendly photosensitisers (PS) sodium magnesium chlorophyllin (Chl) and B17-0024, a synthetic mixture of chlorin e6 derivatives, are compared in terms of photoantimicrobial effectiveness. The use of the anionic Chl requires 1.2% polyaspartic acid sodium salt (PA) as cell wall permeabilising additive. Illumination of samples was performed using a LED array (395 nm) resulting in a radiant exposure of 26.6 J/cm^2. Efficiency testing was done by counting of colony forming units. Illumination after five minutes incubation with 100 µM Chl/PA reduced the count of viable *E. amylovora* ^{WT} by 2.0 x 10^3 and of *E. amylovora* ^{SmR} by 8.0 x 10^5. Extending the incubation period to 30 minutes, increased the photokilling effect towards *E. amylovora* ^{WT} by one order of magnitude, while showing no effect on *E. amylovora* ^{SmR}. Photoactivation of B17-0024 induces the highest bactericidal effect at a concentration of 100 µM and 30 minutes incubation for both strains. Thereby, *E. amylovora* ^{SmR} showed a reduction of viable cells of 1.2 x 10^7 and *E. amylovora* ^{WT} of 1.6 x 10^6. Allover, both strains can be treated effectively with the used PS, whereby *E. amylovora* ^{SmR} is more sensitive to phototreatment than *E. amylovora* ^{WT}. This study proves principle that PDI can be used to treat plant diseases even if causing bacteria are resistant to conventional treatment. [1]

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Giovanni Romano

The role of biofilm optical properties in the modulation of photokilling efficacy: a theoretical model

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In the fields of both antibacterial photodynamic therapy and photoinactivation, the *in vitro* biofilm model is one of the most developed to mimic the *in vivo* conditions. In literature, different illumination geometries and settings are considered to perform photokilling, generally aiming at obtaining uniform irradiance conditions to guarantee the necessary dose reproducibility. Together with irradiation parameters, other physical factors influence the photokilling efficacy once the bacterial strain is fixed. In particular, the biofilm optical absorption at the irradiation wavelength λ_{EXC} can modulate the photokilling inside the biofilm itself, to give the so called "light shading" effect [1]. This means that, for the same impinging dose, the photokilling percentage (efficacy) in an optically thin sample (OD<<1) is expected to be higher than the correspondant percentage in a much thicker one. In fact, a decrease in the light "intensity" (power per unit surface) reaching the deeper layers leads to a decrease of the *local dose* and therefore of the local photokilling efficacy.

In this work, the influence of biofilm absorption on the photokilling efficacy has been modelled and studied theoretically, as a function of the biofilm (BF) optical density OD_{BF} at the irradiation wavelength, corresponding to the photosensitizer main excitation peak. For this purpose, three cases of a plane biofilm were considered, corresponding to $OD_{BF} < 1$, $OD_{BF} \sim 1$ and $OD_{BF} > 1$. The biofilm was represented by a layered model, each horizontal layer being optically thin (OD_{layer} (λ_{EXC}) << 1). The irradiation was modelled as monochromatic ($\lambda=\lambda_{EXC}$) and impinging vertically onto the sample; the dose D was defined as the time integral of the irradiance. The aim of the model was to calculate the expected photokilling efficacy and its dependance on the biofilm OD(λ_{EXC}) by explicitly considering a modulation in the local photokilling rate layer by layer. This could lead to a better comparison between experiments where the main difference lies in the biofilm optical thickness.

[1] Treghini, C., Dell'Accio, A., Fusi, F., Romano, G. (2021). Aerosol-based antimicrobial photoinactivation in the lungs: an action spectrum study. *Photochem. Photobiol. Sci., 20*, 985-996.

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Deeds and misdeeds about sunscreens.

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The growing incidence of skin cancers among the world has attracted the attention not only on the research in the field of theraputic options but also on preventive measures.

Apart from a correct lifestyle behaviour (seeking shade, avoid exposures during the central hours of the days, wear protective dresses), it is important to raise awareness of patients on the correct knowledge and use of sunscreens. There are a lot of sunscreens available on the market, with different kind of filters and action spectrum. It is interesting to note that the incindence of skin cancers has raised concomitantly with the growing market of sunscreens and some concerns have been raised. In addition, in vitro studies have pointed the attention on the possible pollutant effect of sunscreens [1]. Finally, there are concerns about the possible detrimental effects of high SPF sunscreen on vitamin D synthesis [2]. In this presentation I will be discuss these topics in order to shed some light about deeds and misdeeds about sunscreens.

[1] Neale RE et al, (2019). The effect of sunscreen on vitamin D: a review. *Br J Dermato,* 181, 907-915.
[2] D. Fivenson D. et al, (2021). UV filters to protect us: Part 2-Increasing awareness of UV filters and their potential toxicities to us and our environment. *International Journal of Women's Dermatology,* 7, 45–69.

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Antimicrobial PDT: nanoparticles incorporating cyanines and squaraines as new nanophotosensitizers.

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The wide spread of antibiotic resistance is closely connected to the high index of human morbidity and mortality, as well as hospital costs. The developed antibiotic resistant strains of bacteria to conventional antibiotic therapy imply the demand for alternative treatments for infectious diseases. One strategy that may lead to improved antimicrobial treatment is the application of antimicrobial photodynamic therapy (aPDT). Among different PSs, polymethine dyes (PMDs), i.e. cyanines and squaraines, are retaining attention in a wide range of applications of science and technology, including PDT [1] and also in a very few examples of aPDT. The advantage of PMDs over other classes of PSs relies in the possibility of easily tuning their structure to obtain the proper photophysical and photochemical properties for the desired application. However, the highly efficient PMDs are generally hydrophobic causing self-aggregation of the PS under physiological conditions, detrimental in view of an efficient aPDT as it diminishes ROS generation capability. To overcome these drawbacks, a possible solution would be the incorporation of these dyes inside nanoparticles (NPs), which, in addition to preventing the formation of dye aggregates in aqueous media and protecting their photochemical characteristics, have shown an important role in the treatment of infection [2,3].

The present work deals with the design and development of innovative nanophotosensitizers based on different type of NPs incorporating SQs and CYs with different structural features, with potential application in aPDT. NPs-dye complexes were characterized by DLS, NTA, FE-SEM, UV-Vis and fluorescence spectroscopy, exhibiting size ranging between 150 and 200 nm, excellent optical properties and remarkable photostability. In addition, their ability to produce Reactive Oxygen Species (ROS), which could produce bacterial death, was evaluated. The results proved that ROS generation ability is preserved in NPs-dye complexes. Furthermore, in vitro antimicrobial activity studies against Gram-negative and Gram-positive model bacteria in planktonic state were conducted to evaluate the potential of these nanophotosensitizers for aPDT in the local treatment of infections.

1) Dereje, D.M., Pontremoli, C., Moran Plata, M.J., Visentin, S., Barbero, N. (2022) Polymethine dyes for PDT: recent advances and perspectives to drive future applications, Photochem Photobiol Sci, 21:397–419.
2) Vallet-Regí, M., Lozano, D., González, B. and Izquierdo-Barba, I. (2020) Biomaterials against Bone Infection, Adv. Healthc. Mater., 9: 2000310-2000351.
3) Chinigò, G., Gonzalez-Paredes, A., Gilardino, A., Barbero, N., Barolo, C., Gasco, P., Fiorio Pla, A., Visentin, S. (2022) Polymethine dyes-loaded solid lipid nanoparticles (SLN) as promising photosensitizers for biomedical applications, Spectrochim. Acta A, 271:120909-120921.