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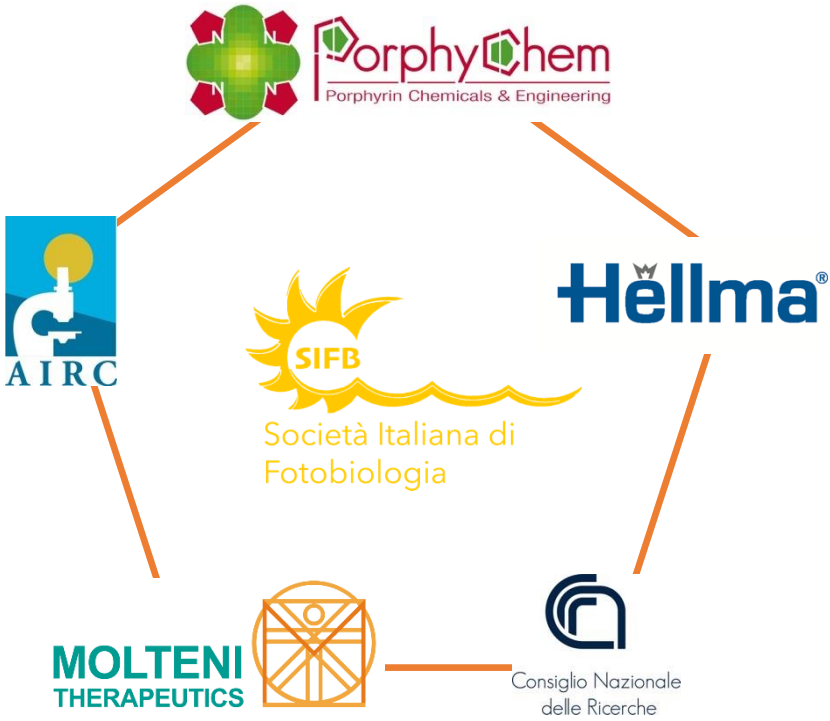

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CONFERENCE PROGRAM

Legend:
PL: plenary; IC: invited communication; OC: oral communication; PC: poster communication

Nanotechnology-mediated PDT

IC1

Plasmonics meets Nanomedicine

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The use of light in medicine impacts the two aspects of the photon that can be seen as an energy quantum as well as a bit of information. In the first case, light is used as a therapeutic agent, directly when biological molecules (nucleic acids, proteins or small molecules such as glutathione) absorb light and undergo a change, or indirectly, when the effect is achieved via an administred photosensitizer (PS) which is the effective light absorber [1]. In the second case, light acts as a diagnostic agent, in particular the nearly noninvasive nature of fluorescence is particularly appreciated by cell biologists, who use fluorescence to image labeled biomolecules in living cells; frequently the used fluorescent probes can be divided into three groups: fluorescent proteins, organic dye molecules and nanosized fluorescent particles, such as quantum dots, nanodiamonds and noble-metal nanoclusters [2].

The optical properties of plasmonic nanomaterials, which arise from the resonance of the oscillation modes of a surface plasma of electrons, are particularly interesting for applications in biology. This phenomenon, known as localized surface plasmon resonance (LSPR), leads to metallic nanoparticles (NPs) absorption of light in a specific region, with a spectral profile that is strongly dependent on their physico-chemical properties and the surrounding environment. The use of engineered nanoparticles can combine the therapeutic and diagnostic aspect of light into a single teranostic agent [3]. The therapeutic action is triggered by the light that induces a local increase in temperature (photothermal therapy, PTT) or the generation of singlet oxygen (photodynamic therapy, PDT): in both cases plasmonic NP is coupled with a PS that harvests incident light and transfers exciton to metal nanoparticles (for PTT) and O2 (for PDT). In the case of highly-luminescent transition metal complex as PS also luminescence is detected, making functionalized gold nanoparticles an example of nanotheranostic device [4,5].

In view of a direct application in medicine, the coupling of the gold NPs with a proper PS creates newsworthy challenges, regarding the optimal conditions for a talking between the NP plasmonic modes and the excited states of the PS, and the necessary correlation with the spectral therapeutic window of living tissues. In this context two approaches can be seen: the first involves the modification of the photophysical properties of the PS, while the second one involves the tuning of the plasmonic properties of the NPs. This talk will present the results obtained in vivo on human glioblastoma mouse xenograft model by treatment with a nanotheranostic device constituted by gold NPs coated by a silica shell doped with an Iridium complex, and the strategies to modify the plasmonic response of the gold core by changing its shape and coating.

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OC1

Intercalation of Bioactive Molecules into Nanosized ZnAl Hydrotalcites for Combined Chemo and Photo Cancer Treatment.

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Hydrotalcites-like compounds (HTlc), also known as layered double hydroxides, represent a class of lamellar solids with net positive charge balanced by exchangeable interlayer anions [1]. HTlc exhibit excellent properties, ability to accommodate a wide range of anionic species with different sizes, biocompatibility, pH-dependent stability and low toxicity, that make them suitable as inorganic drug delivery system for anticancer therapy [2]. Two different molecules were selected for being separately intercalated into ZnAl-HTlc, with formula $[Zn_{0.72}Al_{0.28}(OH)_2]Br_{0.28}{\cdot}0.69\ H_2O$: the anticancer drug norcantharidin (NCTD), known for promoting cell cycle arrest in G2/M phase, and the tetra-sulfonated aluminum phthalocyanine (AlPcS4), a photosensitizer used in photodynamic therapy that can be activated by near-infrared light. The obtained hybrid ZnAl-HTlc, were characterized in terms of X-ray powder diffraction pattern, thermogravimetric analysis, SEM microscopy, drug release profile, *in vitro* cytotoxicity, and ability to produce ROS and $^1O_2$ upon light irradiation. Our data clearly indicate that the two selected compounds are efficiently intercalated within HTlc layers. Remarkably, *in vitro* preliminary studies, performed on a panel of cancer cell lines, indicated a greater cytotoxicity of the two drugs once loaded on HTlc either when administrated singularly or in combination. In addition, the analysis of the synergistic effect of the two formulations was evaluated by determining their combination index, which showed a greater cytotoxicity when using as a 1:2 ratio of AlPcS4@HTlc and NCTD@HTlc, respectively.

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OC2

Nanocomplexes based on a cationic porphyrin and an anionic cyclodextrin with antimicrobial photodynamic properties

Roberto Zagami,[1] James D. Pipkin,[2] Vince Antle,[2] Domenico Franco,[3] Laura De Plano,[3] Salvatore Patanè,[4] Salvatore Guglielmino,[3] Luigi Monsù Scolaro,[1,2,5] and Antonino Mazzaglia[1]

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Nowadays, novel less-expensive nanoformulations for *in situ*-controlled and safe delivery of photosensitiser (PS) against opportunistic pathogens in body-infections areas needs to be developed. Following our ongoing research on nanophototerapeutics [1-3], here we propose the design and characterization of a novel photosensitizing nanosystem based on the marketed cyclodextrin CAPTISOL® (sulphobutylether-beta-cyclodextrin, SBE-beta-CD) to fabricate efficient biocompatible systems for antimicrobial photodynamic therapy (aPDT). Firstly, interaction studies were carried out in order to investigate the complexation between CAPTISOL® with the tetracationic water soluble meso-tetrakis(N-methylpyridinium- 4-yl)porphine (TMPyP). Nanocomplexes based on CAPTISOL® and TMPyP (NanophotoCapitsol) were prepared in aqueous media and characterized by complementary spectroscopy and microscopic tecniques such as UV/vis, fluorescence spectroscopy, atomic force microscopy (AFM) and scanning-near field optical luminescence (SNOL), thus investigating complex stoichiometry, stability constant, photophysical and morphological properties. Furthermore size and ζ-potential were measured by Dynamic light scattering and Electrophoretic Light Scattering, respectively. Release and stability studies were performed in physiological conditions pointed out the role of CAPTISOL® to sustain the PS release. Finally, photoantimicrobial activity of the NanophotoCapitisol *vs* free porphyrin were investigated against Gram-negative *Pseudomonas aeruginosa* ATCC 27853, by showing as the proposed nanosystems can control along the time the release of porphyrin to photokill Gram-negative bacterial cells.

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Light-responsive materials

IC2

Structral Colours and Light management in Algae.

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Nature's most vivid colours rely on the ability to produce complex and hierarchical photonic structures with lattice constants on the order of the wavelength of visible radiation [1]. Structural coloration is widespread in the marine environment. Within the large variety of marine organisms, macroalgae represent a diverse group of more than 24 000 species. Some macroalgae have developed complex optical responses using different nanostructures and material compositions [1]. In this talk, I will describe the mechanisms that are employed to produce structural color in algae and provide a discussion on the functional relevance by analyzing the geographical distribution and ecology in detail. I will discuss on how structural color is influenced by local factors such as radiation intensity and turbidity of the water.

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OC3

Light propagation through colloid-polymer mixtures: towards uniform irradiance sources for phototherapy applications

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Phototherapy is a widespread method for treating several dermatological diseases such as acne, vitiligo, and psoriasis. One of the main drawbacks of conventional light sources for phototherapy is that they are not uniform in their output. Non- uniform illumination of the skin treatment area is not desirable, in fact the risk is to have insufficient radiation in some points and dangerously excessive in others. Emission uniformity of light-emitting sources is a very desirable property also for *in vitro* experiments to measure e.g. PDT efficacy studies in the case of new photosensitizers and/or photosensitizer carriers, to keep radiant exposure at the sample level (Watt/cm^2) as much constant as possible.
In order to obtain a uniform light emission, we developed a diffusing gel in the visible-UVAUVB bands. We used two biocompatible materials for gel synthesis: Intralipid® and methocel. Intralipid® is composed of soy fat droplets (with a mean diameter of 0.5 micron) and egg yolk phospholipids suspended in water, used for parenteral nutrition; due to its light diffusion properties, it is used as a light scattering medium for tissue phantom studies. Methocel is a cellulose-derived polymer, with a good transparency across the whole UV-visible spectral range and stable over time. By dispersing Intralipid into methogel we have tried to obtain a diffusing, yet non liquid thus more easily handable material, to further define a gel-like illuminator, whose light-diffusion properties are exploited to obtain a uniform illumination source for phototherapy. Starting from the definition of the best protocol to obtain transparent methocel at different concentrations, we have studied light propagation through colloid-polymer mixtures by varying both the Intralipid® concentration and the material thickness. To this aim, light has been injected in the mixture by external UVA illumination (metal-vapour and LED sources), undergoing scattering by the Intralipid® component. To quantify light emission by the mixture (e.g. radiant exposure), we used a Gafchromic® EBT3 film dosimeter [3], whose 2D darkening response was analysed by film scanning and subsequent image analysis methods to convert scanned images from films into radiant exposure maps of the light emitted by the mixture and received by an illuminated surface. The obtained illumination profiles were correlated with the injected light parameters, mixture thickness and Intralipid® concentration. The obtained results show that emission uniformity increases at both increasing thickness and concentration, accompanied on the other side by a decrease in radiant exposure.

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OC4

Photoluminescent iridium(III) complexes functionalised with a cationic triphenylphosphonium side chain

Eleonora Bettalico[1,2], Matthew Stout[2], Luca Rigamonti[1], Erika Ferrari[1], Massimiliano Massi[2]

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A family of four tetrazolato ligands functionalised with a triphenylphosphonium lateral chain and the corresponding iridium(III) complexes were obtained through different synthetic steps and characterised via ^{1}H, ^{13}C and ^{31}P NMR and UV-vis spectroscopies. The compounds are distinguished by variations of the aryl substituent on the tetrazolato ligands. The photophysical proprieties of these derivatives were evaluated in organic media, revealing an emission predominantly arising from mixed metal-to-ligand and ligand-to-ligand charge transfer excited states of triplet multiplicity. The functionalisation with the triphenylphosphonium salt causes a systematic red-shift of the emission profiles, comparing to the corresponding neutral and methylated complexes previously reported. The luminescence proprieties of these probes can be modulated through a specific alkylation of N1 or N2 atom of the tetrazole ring. The lipophilicity and the behaviour of complexes with different alkylating position were also examined through *in vitro* tests.

OC5

Hybrid photosynthetic enzymes as versatile photoactive soft materials.

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The complexity of the natural photosynthetic systems is difficult to reproduce *in vitro*; however, complexity is inherently associated to the efficiency of the living multienzyme character of photosynthesis and any biomimetic attempts must cope with this stringent requirement.
In this regard, we have designed and assembled efficient organic-biological hybrid systems formed by small to medium size organics molecules responsible of a given specific role and the photoenzyme responsible for energy transduction in photosynthetic organisms.
Applications of photoresponsive enzymes as soft photoconverting material in different environment will be presented to show drawbacks, limitations and potentials of such hybrid systems, along with some future interesting developments.

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OC6

UV-light Sensitive Visible and Near-Infra Red Emitting Lanthanide tris(β-diketonate) Complexes and their Optoelectronic Applications

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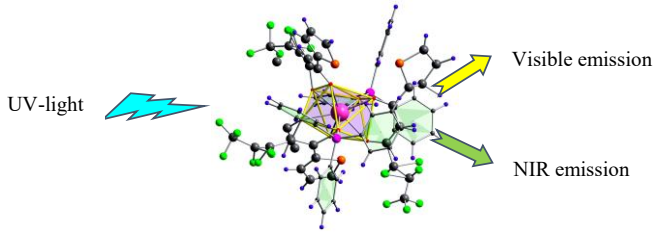
UV-light sensitive visible- and near-infrared (NIR)- luminescent lanthanide (Ln) complexes are used in an impressive number of applications, such as sensors [1,2], bioassays [3], or OLEDs (organic light-emitting diodes) [4], owing to their peculiar photophysical properties. Such complexes with organic ligands, of high absorption coefficients, show excellent photoluminescence quantum yield (PLQY) and stability. Regarding the strategies to enhance the PLQY, the most common is the suppression of the radiationless deactivation pathways due to the presence of high-frequency oscillators (e.g. –OH, –CH groups) around the Ln centre. Recently, a different approach to maximize the PLQY of Ln(β-DKs) has been proposed (named "Escalate Coordination Anisotropy", ECA). It is based on the assumption that coordinating the Ln ion with different ligands will break the centrosymmetry of the molecule leading to less forbidden transitions (loosening the constraints of the Laporte rule). Such complexes are very interesting for biomedical applications [1,4]. Literature reports many OLEDs based on such complexes, but with low efficiency and stability. Consequntly, there is a need to develop some new Ln complexes with enhanced PLQYs and stabilities to get efficient and stable devices. For this purpose, UV-light sensitized Ln(III) complexes with various fluorinated/non-fluorinated β-diketones and O/N-donor neutral ligands were synthesized. The complexes were characterized thoroughly and their photophysical properties were studied to select the best ones for the fabrication of stable and efficient OLED. Finally, the OLEDs were fabricated and investigated using these complexes as emitting layers along with other organic layers like NPB (hole-transporting layer), BCP (hole-blocker) and Alq_3 (electron-transporting layer). The devices show strong red (612 nm) and near infra- red (998–1064 nm) electroluminescences corresponding to characteristics transitions of Eu(III), Yb (III) and Nd(III) ions.



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Light-induced therapies (infections & dermatology)

IC3
Overview of photodynamic therapy and immune response

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Photodynamic therapy (PDT) is an oncology clinical treatment option that is characterized by the combined use of light and a photosensitizing agent, called photosensitizer, which in turn produces a cytotoxic event able to kill cancer cells. Indeed, the PDT therapeutic effect is mediated by the reactive oxygen species (ROS) and by the singlet oxygen that are formed in the presence of molecular oxygen and upon irradiation of the photosensitizer with light at a specific wavelength.
Moreover, the cells damage produced by PDT is even more rapid thanks to the concomitant microvascular degeneration and the induction of apoptotic, inflammatory and immunological mechanisms. PDT can initiate membrane-level events involving transduction signals. This includes an increase in the expression of stress proteins and activation of genes that regulate apoptotic processes. Thanks to their role in the processes of cell adhesion and antigen presentation, some PDT-induced stress proteins may be involved in the development of an immune mediated response that occurs during photodynamic therapy. A remarkable inflammatory reaction is one of the essential events in the PDT-induced tumor destruction mechanism by releasing a wide variety of mediators such as vasoactive substances, components of the complement cascade, cytokines (Il-6, Il-2, TNF, etc.), growth factors and other immunoregulatory factors. Therefore, PDT is able to generate a remarkable "***Antitumor Immunity***" by activating cytotoxic T lymphocytes and the combination of these two approaches might represent a promising option for treating against aggressive and metastatic tumors.

OC7

Blue LED light effects in cultured human keloid fibroblasts

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Keloids are wounding-induced fibroproliferative human tumor-like skin scars of poorly defined pathogenesis [1]. These scars are characterized by an exaggerated synthesis of collagen probably due to the increase of fibroblasts density and their proliferation rate [2-3]. Currently, keloid treatment can be divided into three main categories: non-invasive medical therapies, surgical and other invasive therapies, and new therapies under investigations [4]. About the last choice, physical treatments are recently proposed and new technologies and devices have been developed [5].

In our study, we used a Blue LED light (410-430nm, 22 J/cm^2) to conduct *in vitro* experiments: 11 human keloids and 7 boundary tissues from 10 patients were used to perform fibroblast primary cultures which were irradiated at different treatment times in the range 5÷60s. Two colorimetric tests (Cell Counting Kit-8 and Sulforhodamine B based assay) were performed to study cell metabolism and proliferation. 168 tests at 24h and 112 tests at 48h after irradiation were executed. Raman spectroscopy was used to study eventual direct effects on cytochrome C. We also investigated possible effects of Blue LED light on membrane currents correlated to cell cycle modulation with patch-clamp recordings. 24h after irradiation, a significant reduction of metabolism was observed in the samples with the following irradiation times: 20, 30, 45 and 60s (n=63), without changes in cell proliferation (n=62). At 48h, the decrease in cell metabolism was confirmed (n=41) and accompanied by a decrease in cell proliferation (n=42). Electrophysiological recordings showed an enhancement of voltage-dependent outwards currents activated by a depolarizing ramp protocol (from -80mV to +80mV; 800ms) after a 30s irradiation; n=18. The effect peaked 3min after irradiation and was reverted to baseline levels in about 5 min. Blue LED light irradiation directly affects human keloid fibroblasts: it decreases cell metabolism and inhibits membrane currents. This treatment could represent a non-invasive approach in the management of hypertrophic scars and keloids.

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OC8

Photoinactivation of *Pseudomonas aeruginosa* biofilm by blue light

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Pseudomonas aeruginosa is an opportunistic pathogen that can cause severe nosocomial infections in different body districts, including wounds, ulcers and urinary tract. This microorganism takes advantage of a combination of resistance mechanisms to overcome the action of antimicrobials and, as a result, infections become difficult to treat, especially when *P. aeruginosa* grows as biofilm [1]. Within recent anti-*Pseudomonas* approaches, antimicrobial Blue Light Therapy (aBLT) gained increasing interest. aBLT is based on the effect of visible light, particularly in the region from 390 to 500 nm, to control the bacterial growth and biofilm formation of a broad-spectrum of pathogens, including bacteria, yeasts and fungi [2]. The mechanism of action is not fully understood. It has been hypothesized that endogenous photosensitizers may induce photo-oxidative stress upon irradiation causing photo-oxidation of microbial macromolecules and cellular death, as a consequence [3].
In this study, blue light at 410 and 455 nm were used to inhibit and/or eradicate biofilm of *P. aeruginosa* PAO1, chosen as model microorganism. A multi-well plate was used as *in vitro* setup. Crystal violet staining of adherent biofilm, combined with cell viability of planktonic and sessile populations, permitted to evaluate the effect of blue light on cells and matrix. Confocal microscopy analyses have been also performed to evaluate the efficacy of aBL.
Upon increasing radiant exposures, blue light at 410 nm successfully inhibited biofilm formation of *P. aeruginosa* PAO1, causing a significant decrease in adherent biomass and cell viability of adherent and planktonic phases. Blue light at 455 nm showed a very good inhibitory effect. Fifteen *P. aeruginosa* strains isolated from catheters-associated urinary tract infections, characterized by a different ability to form biofilm, were sensitive to aBL. Moreover, blue light at 410 nm was also active in eradicating young and old biofilms of PAO1 strain. Interestingly, blue light seems to affect the ability to form matrix. Further investigations are needed to evaluate how blue light damages biofilm machinery.
Blue light at 410 nm is effective in inhibiting and eradicating *P. aeruginosa* biofilm in a dose-light dependent manner. This approach could be exploited in different applications in which *P. aeruginosa* growth control is needed, such as clinical, environmental and industrial fields.

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PL1

Dye Doped Silica Nanoparticles as Organized Systems for Nanomedicine

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Silica nanoparticles are versatile platforms with many intrinsic features, including a low toxicity. Proper design and derivatization yield particularly stable, very bright nanosystems displaying multiple functions,[1] which can be used for either otpical and photoacoustic imaging[2] and for photoluminescence (PL) and electrochemi-luminescence (ECL) sensing.[3] In addition, silica nanoparticles can also be used for as platforms for phto-dynamic and photo-thermal therapies.[2] For these reasons, silica nanoparticles already offer unique opportunities, and further improvement and optimization can substantially expand their possible applications in fields of high impact, such as medical diagnostics and therapy, environmental and food analysis, and security.

In this context, we have developed a direct micelle assisted strategy based on the use of Pluronic F127 as high molecular weight surfactants. The one-pot synthesis yields PEGylated silica nanoparticles endowed with very high monodispersity, colloidal stability and core-shell structure. These nanoparticles were recently reported with the acronym PluS NPs (Pluronic Silica NanoParticles). These NPs had a silica core of about 10 nm and an overall hydrodynamic diameter of about 25 nm. Interestingly, PluS NPs can be tailored for optimization of processes such as directional energy transfer, which provide those systems with extremely valuable functions: high light-harvesting capability, signal-to-noise maximization, multiplex output, and signal amplification. *In-vivo* experiment proved the absence of toxic effects on mice even after three months after injection. We also found that cellular uptake was influenced by nanoparticle functionalization while the drug loading ability can be tuned with a suitable choice of the silica precursor.

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Light-induced therapies (cancer)

IC4

Light-controlled delivery of cancer immunotherapeutics.

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Cancer immunotherapeutics such as immunotoxins and peptide-based cancer vaccines are taken up into cells by means of endocytosis. The majority of these drugs are subsequently sequestered and degraded in late endosomes and lysosomes. This resistance mechanism results in poor anti-cancer activities of immunotoxins and weak CD8+ T-cell responses after therapeutic cancer vaccines. Thus, there is a need for drug delivery methods which can improve the endosomal escape of immunotherapeutics having intracellular targets.

Photochemical internalization (PCI) is an intracellular drug delivery method based on light-activation of lysosomotropic photosensitizers followed by ROS-generation and a subsequent membrane-disruption of endosomes and lysosomes, leading to cytosolic release of the entrapped drugs of interest. PCI has been found safe and tolerable in one clinical trial [1] and is currently under evaluation in to other clinical trials; one in combination with gemcitabine, followed by systemic cisplatin/gemcitabine, for the treatment of inoperable cholangiocarcinoma [2] and one for the PCI of vaccine antigens (HPV peptides and KLH) for induction of B- and T-cell immune responses in combination with the adjuvant poly-ICLC (Hiltonol) [3].

The overall aim of our project is to develop and explore PCI as a rational strategy to enhance intracellular release and efficacy of (1) immunotoxins targeting cancer stem cells (CSCs) and (2) therapeutic cancer vaccines.

In this presentation, fimaporfin (TPCS2a)-based PCI of immunotoxins targeting CSC markers such as CD133, CD44, CSPG4, EpCAM and CD105 (Endoglin) will be demonstrated. In addition, cancer cells over-expressing stem cell markers important for detoxification such as ABCG2 (BCRP/CD338), ABCB1 (P-gp/MDR1) and ALDH are highly sensitive to photochemical treatment using PCI photosensitizers. Mechanistic evidence showing that PCI strongly enhance MHC class I presentation of peptide vaccine antigens important to mount robust CD8+ specific antitumor responses will also be provided.

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[2] ClinicalTrials.gov Identifier: NCT02947854

[3] ClinicalTrials.gov Identifier: NCT01900158

OC9

Targeting tumor cells with photosensitizer-protein complexes.

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Bioavailability of photosensitizers for cancer photodynamic therapy is often reduced by their low solubility in water. Proteins can be exploited to overcome this issue and deliver photosensitizing molecules to target cells in high yield. We have bound several naturally occurring photosensitizers to proteins either through hydrophobic or covalent interactions, achieving fully functional photosensitizing systems that proved effective against bacteria [1-6] and cultured tumor cells. In this work we show that proteins can be engineered or chemically modified to comprise a targeting sequence or domain, that confers selectivity towards specific cell lines. In particular, we report the development of a genetic construct that targets the human prostate cancer cell line PC3 and is able to deliver a highly efficient photosensitizer. Fluorescence emission by the photosensitizing molecules allows tracking the location of the construct and assessing its effectiveness in targeting of tumor cells.

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OC10

Novel non-symmetrical diaryl porphyrins inhibit cellular proliferation and migration of human cancer cell lines.

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Various classes of photosensitizers, many of which include the cyclic tetrapyrrole core structure typical of porphyrins, are currently in clinical use or at different stages of preclinical and clinical development for photodynamic therapy (PDT) of cancer. However, the available PSs are less than ideal for clinical use, due to uncomfortable adverse effects, such as phototoxic and photoallergic reactions, or suboptimal tissue penetration, and research for novel PSs featuring more favorable properties is ongoing. Recently, it has been shown that mixed non-symmetrical diaryl porphyrins, with two different pendants, are more photodynamically active than symmetrical diaryl porphyrins.

In the present study, we investigate the *in vitro* photodynamic effects of four novel non-symmetrical diaryl porphyrins, two of which bear one pentafluoro-phenyl and one bromo-alkyl (apolar) pendant, whereas the two others bear one pentafluoro-phenyl and one cationic pyridine pendant. The effect of the four compounds on cell viability (in 2D and 3D cell culture), along with their cellular uptake, and their ability to induce apoptosis, necrosis and/or autophagy and to inhibit spontaneous cell migration were evaluated on a small panel of human cancer cell lines and compared with the properties of m-THPC (Foscan), currently the most successful PS approved for clinical use in cancer PDT.

The results of the cytotoxicity studies indicate that the two molecules bearing the cationic pendant are more potent *in vitro* than those with the apolar pendant, and that they are as potent as Foscan. Furthermore, a greater potency of diaryl porphyrins with a positive charge in inducing cell death, as compared to those with the bromo-alkyl pendant, has also been observed; most importantly, some of these novel compounds exhibit features that might make them superior to the clinically approved PS Foscan.

OC11

Does RNASET2 positively influence PDT-induced oxidative stress?

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Introduction. Photodynamic therapy (PDT) is a highly selective and low-invasive therapy for the treatment of solid tumors. The PDT involves the use of a photosensitizing molecule (PS) in association with light of an appropriate wavelength. In the presence of molecular oxygen (O_2), following a series of energy transfers, reactive oxygen species (ROS) are produced, among which there is also the singlet oxygen ($^1O_2$). This species of oxygen has a high level of cytotoxicity and causes most of the cell damage induced by PDT. ROS produced in this way leads to cell death by apoptosis, necrosis or autophagy. In several cell lines, the *RNASET2* gene is correlated to an increase in mortality following the induction of stresses such as lack of amino acids or hypoxia [1]. Thus, this work aims to verify if *RNASET2* could also influence the stress induced by PDT.

Material and Methods. To this purpose, OVCAR-3 cells (deriving from ovarian adenocarcinoma) were used, which differ in the expression of the gene in analysis (expressing the *RNASET2* gene and its silenced counterpart, which is characterized by a reduced expression of the gene under study). In addition to this, the effect of the recombinant *RNASET2* glycoprotein (deriving from *Pichia pastoris*), added to the medium at different concentrations and time, has also been verified. The tests were performed with a PS belonging to the BODIPY family, compounds generally used as fluorescent dyes [2] that can be modified with the introduction of iodine atoms thus becoming an alternative class of PSs [3], which has recently found application in photodynamic therapy [4]. To gain some insights into the mechanism of PS-induced phototoxicity, induction of apoptotic, autophagic and necrotic cell death, and generation of reactive oxygen species (ROS) were evaluated in cancer cells following exposure to the PSs and irradiation. The effect of the PSs on the migratory activity of the cells was also assessed.

Results and Discussion. The results obtained confirm that the *RNASET2* gene leads to an increase in cellular mortality under the stress induced by PDT; however this result was not replicable following the addition of the recombinant glycoprotein in the cell culture.

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OC12

PBL (Psoralens + Blue light): blue light activates 8-MOP and TMA triggering vesical (T24) tumor cell apoptosis and death.

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BACKGROUND. Psoralens and angelicins (furocoumarins) are natural and synthetic compounds with high antiproliferative potency under UVA irradiation mainly used for the treatment of skin diseases (PUVA therapy) or immunological disorders in extracorporeal photopheresis (ECP). To improve their activity against psoriasis or vitiligo and avoid severe side effects mainly related to the formation of interstrand crosslinks (XLs) with DNA pyrimidine bases, a variety of derivatives, hopefully monofunctional, have been synthesized. Although angelicins, due to their angular geometry, do not generally form XLs, some of them, i.e. (TMA), can crosslink folded DNA upon UVA. Furthermore, furocoumarins produce ROS that impair cellular functions through lipid peroxidation, oxidation of guanine and strand breaks in nucleic acids, oxidation of proteins and inactivation of enzymes.
To photoactivate 8- MOP and 4,6,4'-trimetylangelicin (TMA) towards bladder (T24) cancer cell lines, a new approach based on less toxic and more penetrating visible radiation (BL, 420 nm) is proposed.
RESULTS. TMA and 8-MOP showed high antiproliferative activity towards cancer cells, through induction of apoptosis. Besides ROS generation (less efficient under BL than UVA), the proapoptotic effect seemed related to the activation of p38 and inhibition of p44/42 phosphorylation. Moreover, no phosphorylation of the histone H2AX, nuclear β -catenin and GSK3β occurred. Moreover, Cyclin D1, c-Myc and CD44v6 expression were reduced through inhibition of the Wnt pathway. Overall, with respect to previous experiments, our compounds appeared a little less sensitive to PBL vs T24 than vs prostate cancer cells (DU145), showing a specificity of the test compounds towards different tumor cell lines. The strong photocytotoxicity of TMA and 8-MOP can be related to the kind and number of DNA lesions. Under BL, no mutagenic crosslinks, no photocleavage nor photooxidative lesions were detected on isolated DNA by TMA phototreatment, but only MAs can form. However, generation of XLs still remained for 8-MOP under BL but in a lower amount than under UVA.
CONCLUSIONS. Overall, our results indicate that 8-MOP, and particularly TMA, can be efficiently activated by BL and may be considered good candidates for targeted PBL of bladder cancers and possibly for other solid tumors.

Light-responsive materials

IC5

Photoactive nanomaterials for medical applications.

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Highly efficient antimicrobial polymer nanofiber membranes and nanoparticles that use inactivation of a pathogen via photosensitized generation of singlet oxygen will be presented. The antimicrobial mechanism includes excitation of the photosensitizer encapsulated or externally bounded to nanomaterials by visible light, formation of its triplet states followed by energy transfer to triplet oxygen leading to singlet oxygen formation. Since singlet oxygen is a great oxidant of biological targets, the antibacterial and antiviral effect is very powerful. The singlet oxygen-sensitized delayed fluorescence of a photosensitizer can be observed and used as a sensitive tool for detection of oxygen, imaging of singlet oxygen and distribution of a photosensitizer [1]. Modifications of nanomaterials with NO-photodonors photoproducing NO radical with the aim to increase their antimicrobial effect will be also mentioned [2].

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OC13

Heterocomposites from diatoms microalgae.

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Diatoms, a brown type of microalgae, spontaneously uptake silicic acid from the ocean and build highly porous biosilica structures, starting from silica-derived pool vesicles. These nanotextured hydrated biogenic silica structures naturally self-assemble at mild conditions, conversely the industrially produced silica. All the biosilica production occurs in coupling with photosynthesis. Quite recently, diatoms gained more attention in different fields like photonics, sensing, optoelectronics and biomaterial science [1], since their silica shells, called frustules, can be chemically decorated *via* silane surface reaction [2] or biochemically functionalized *via* in vivo feeding with small molecules. Then facile extraction methods enable the collection of functionalized silica structures directly from fed diatoms. Here we present green production of phosphorescent nanoparticles from living *Thalassiosira weissflogii* diatoms fed with a luminescent cationic Ir-complex [3], together with a variety of emissive silica structures extracted using *in vivo* or *in vitro* manipulation of diatoms with new synthesis fluorescent dyes; these resulting functionalized silica walls were used for photonics [4] and silica-based singlet oxygen generation in the Photodynamic Therapy field. We also managed to produce biosilica functionalized with pharmacological moieties in order to produce natural ensembles, which resulted useful as bidimensional scaffold for bone tissue engineering. [5] A last remarkable approach of functionalization is based on the coating of both living diatoms and their shells with polydopamine (PDA) to produce metal-based heterostructures [6] or for incorporating enzymes during dopamine polymerization. In conclusion, orthogonal methods for functionalization of biosilica from algae lead to proficient perspectives of manufacture of new generation materials for bioelectronics or biomedicine.

Acknowledgements

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OC14

Naphthalene diimides, a versatile platform for biomedical applications

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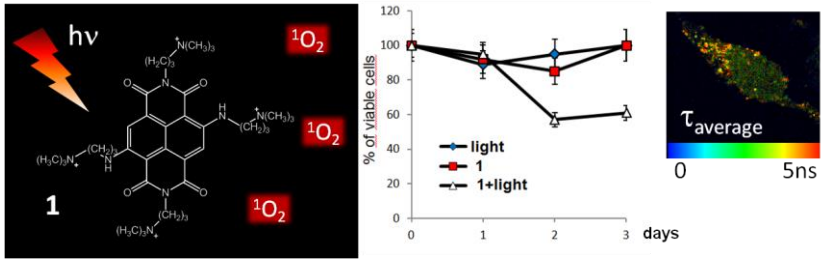
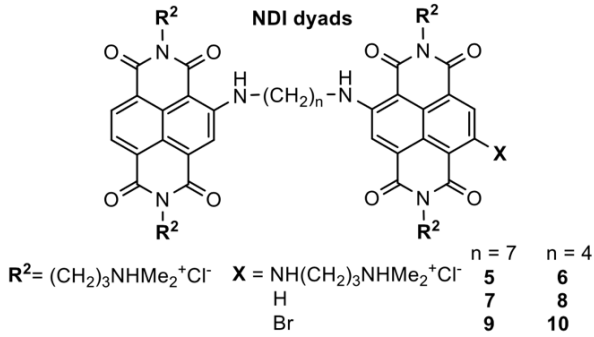
Naphthalene diimides (**NDIs**) are extremely versatile compounds. Their optoelectronic properties can be effectively tuned by substituents on the aromatic core, thus giving origin to absorption and emission in the red spectroscopic window, which makes them appealing for fluorescence imaging and photodynamic therapy (PDT).[1] Additionally, thanks to their chemical accessibility and large planar surface NDIs have been explored as appealing scaffolds for the design of RNA and DNA G-quadruplex (G4) ligands. We have shown that tri-, and tetra-substituted NDIs are potent and reversible ligands targeting G-rich nucleic acids (NAs) folding into G4s. Recently, we have published a series of NDIs having excellent water solubility and cellular entry, merged with promising features for theranostic applications. In particular the tetra-substituted NDI compound **1** was able to produce singlet oxygen, and induced photo-cytotoxicity.[2] Nuclear uptake was evidenced by fluorescence confocal imaging exploiting the intrinsic NDI fluorescence. (see figure)



Lately we investigated NDI dyads, see scheme below, for their potential as anticancer drugs and light-responsive systems to sense G4 DNA.[2] We will briefly illustrate the biophysical and biological results obtained with these DNA ligands based on the NDI platform.



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IC6

Silk fibroin hydrogels as potential drug delivery system in photodynamic therapy

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The number of suggested biomedical applications of silk fibroin (SF) grew steadily in the recent years.[1] The properties of SF (e.g., mechanical robustness, biocompatibility, biodegradability, optical transparency) and its versatility to be processed into diverse forms (e.g., films, sponges, hydrogels, microparticles, microneedles etc.) made it an appealing material for regenerative medicine applications and as a component of medical devices. In addition, SF hydrogels lend themselves to the incorporation and release of biologically active compounds and have been explored at matrices for drug delivery systems.[2] SF has been used for the controlled delivery of various therapeutic agents, but to the best of our knowledge, the SF-mediated delivery of photosensitizers has not been explored to date. Photosensitisers are the drugs used in photodynamic therapy (PDT), a clinically approved modality that achieves selective destruction of target cells/tissue via the localised light-triggered generation of reactive oxygen species (ROS). PDT has been successfully employed to eradicate malignant/premalignant tissue, microbial infection, and for wound healing, but its applicability has been hampered by the poor hydrophilicity of most photosensitisers.[3] In this study, SF hydrogels were used as a matrix to incorporate a porphyrin photosensitizer for application in photodynamic therapy (PDT). The hydrogels obtained were characterized by rheology, spectrophotometry, and scattering techniques to elucidate the factors involved in the formation of the hydrogel, and to characterize the behaviour of silk fibroin (SF) after incorporating of the porphyrin to the matrix. The hydrogels displayed a shear thinning behaviour and were able to recover their structure over time after shear deformation. Crucially, the formation of self-assembled peptide nanostructures prevents porphyrin aggregation, thereby increasing the generation of ROS. Our findings suggest that porphyrin can diffuse out of the hydrogel and permeate the outer skin layers. This evidence suggests that SF hydrogels can be used as media for porphyrin encapsulation and drug carriers for the sustained release of photosensitizers for PDT.

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Poster Session

PC1

Bioconjugation strategies in garnishing the bacteria photosynthetic reaction center.

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Bioconjugation is synthetic technique that covalently links proteins, commonly involving cysteine, tyrosine and lysine residues, with other biomolecules or synthetic ligands such as dyes or polymers. The aim in our research group is the manipulation of photosynthetic enzymes, responsible for the conversion of solar light in other viable forms of energy, with the purpouse of extending their photoactivity to non-conventional applications *via* bioconjugation.

The focus is concentrated on the photosynthetic reaction center (RC) from the wild type and the carotenoidless strain of the purple bacterium *Rhodobacter sphaeroides*, a membrane-spanning protein composed of three subunits, nine co-factors, and 22 possible targeting lysine. Bioconjugation to lysine groups (Figure 1a) needs few specific requirement, including: i) protein with available lysine; ii) ligand with a carboxylic group to be activated in the corrisponding succinimidyl ester; iii) non-denaturating operating condition to avoid protein unfolding, such as acqueous buffer with a percentage of surfactants, dark envioriment during the reaction and green lighting in the following steps of purification and characterization. This strategy has been used to obtain hybrid system with *ad-hoc* synthesized tailored organic molecules that function as artificial antennas (Figure 1b) to enhance the light harvest ability of pristine RC.

We are currently developing further one-step "grafting-to" bioconjugation protocols to assemble covalent system encompassing other peculiar molecules and biomolecule, including small proteins. A specific case of protein-protein bioconjugation will be presented.

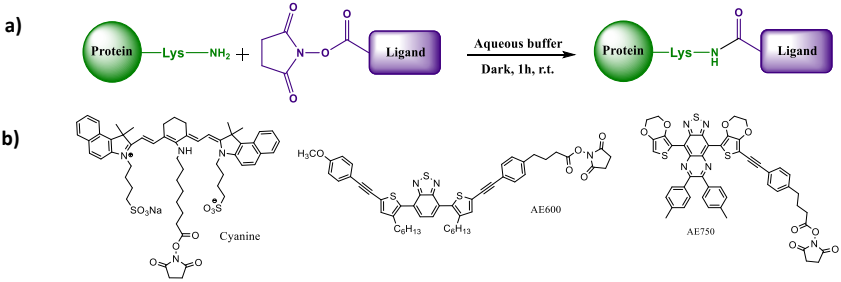

Figure 1. a) Scheme for a generic ligand-protein bioconjugation reaction; b) Example of antennas previously bioconjugated with the bacterial RC

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PC2

Combined PDT and doxycycline against *Helicobacter pylori*: indications of a synergistic and non-toxic effect

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In recent years, the emergence of an increasing number of multidrug resistant *Helicobacter pylori* -associated infections leads to the urgent search for novel therapeutic solutions [1, 2]. In this regard, different PDT treatment strategies have been proposed, all characterized by the absence of external photosensitizers, due to endogenous production of photoactive porphyrins by *Helicobacter pylori* (*H. pylori*) itself, notably protoporphyrin IX (PPIX).
In this work the possible synergy between doxycycline and therapeutic light was investigated in three different *H. pylori* strains (ATCC 700392, ATCC 43504 and ATCC 49503) susceptible to this antibiotic. Moreover, to evaluate the possible side effects of this therapeutic treatment, the cytotoxicity of this combination with and without PPIX on AGS cells (ATCC CRL-1739), was evaluated [3].
Bacterial cultures were grown on solid medium either containing or not doxycycline at sub-inhibitory concentrations, and irradiated for 10, 20, 30 minutes with a 400nm-peaked light source (4.8 mW/cm^2). Viability was evaluated by post-treatment CFU counting. The phototoxicity tests on AGS cells were performed incubating with or without doxycycline for 72 hours at the above-mentioned concentrations and subsequently overnight with or without 50 nM of PPIX, a concentration higher than the estimated amount of PPIX released *in vitro* by *H. pylori* in culture medium (12-42 nM, literature data). Irradiation was performed with the same parameters used with *H. pylori* cultures and post-treatment cell viability was evaluated by MTT assay. Controls corresponding to irradiated cell samples only were prepared for comparison. Indications of an antibacterial synergistic effect were obtained when both antibiotic and light treatments were performed, showing an enhancement of the photokilling efficacy. No significant toxic effects in AGS cells were observed using PDT, doxycycline and PPIX alone and in combination between them under the same conditions of exposure.
Based on these encouraging results, we can conclude that the combination of doxycycline and PDT against *H. pylori* strains could be considered an interesting therapeutic option associated with no toxicity for the healthy gastric mucosa.

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PC3

New PS derivated from 5,10,15,20-tetrapentafluorophenylporphyrin applied on photodynamic therapy.

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Photodynamic therapy (PDT) is an alternative or adjuvant treatment to classical cancer therapies which achieve the goal to kill cancer cells by using non-toxic drugs or dyes (photosensitizers) that are pharmacologically active only after exposure to light in the presence of oxygen [1]. The four mainly used molecular structures of photosensitizers belongs to the class of porphyrin, chlorins, phthalocyanines, and porphycenes derivatives [2].
The commercially available tetrapentafluorophenylporphyrin has been used as parent compounds for the synthesis of six new tetraarylporphyrins. These new porphyrins were isolated as pure compounds after column chromatography purification, following nucleophilic substitution of the *para*-position fluorine by means of oxygen and sulphur anion, providing either tri- or tetra-substituted derivatives. Of these new porphyrins, were first determined the photobleaching stability and the octanol/water repartition values (LogP), and then were studied as photosensitizers (PSs) against HCT116 cancer cell line irradiating with a blue LED device.
The intrinsic toxicity of all these compounds was negligible whereas the photodynamic efficacy was found related to the hydrophilicity of the tethered moiety as the hydroxy substituted compound was found to be the more efficient compared with the methoxy substituted derivatives. On the contrary, the PSs lacking of any polar groups were found poorly efficient.

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PC4

Fluorescent probes from phage display for myeloma molecular mapping

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In this study, we introduce a proof of concept for an antigenic mapping of cells from hematological neoplasm using phage peptides selected by Phage Display. Experimental plan consisted in the screening and subsequently selection of random M13-pVIII-9aa peptide library against hematological neoplastic cells [1]. For each phage clone, amino acid sequence of foreign peptide has been deduced and compared for mimotopic homology with known proteins related to hematological neoplasm. Phage clones, displaying the foreign peptides with the highest value of deduced homology, have been labeled with isothiocyanate fluorescence and tested in ex-vivo samples by fluorescence imaging technique [2, 3].
By approach, the current state, three different phage peptides has been identified for its ability of binding/discrimination of myeloma plasma cells from healty ones in aspirated bone marrow of two patients affected by multiple myeloma IgA Kappa. Specifically, the phage clones named CLL-IV and EIII-14 showed fluorescence positivity to myeloma cells CD45-/CD38+/CD138+, while EIII-10 phage clone toward those CD45+/CD38+/CD138-. A further phage peptide, previously tested against chronic lymphatic leukemia, revealed a recognition trend of CD138- plasma cells, indiscriminately from the presence/absence of other CD antigens, such as CD45 and CD56.
In the diagnostic and therapeutic field, the comparison of different antigenic profile provides key information about molecular mechanisms of neoplasm pathogenesis and drug-resistance. At this purpose, phage peptides with different binding specificity could be used for the simultaneous discrimination of several subclasses of myeloma cells and the development of targeted therapies.

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PC5

Light-dependent nanoparticles biosynthesis by the freshwater microalgae *Chlamydomonas reinhardtii*.

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In the last decade, nanoparticles are emerging for their commercial importance and applications. Their wide applications have led to numerous methods being developed for the synthesis of nanoparticles of various shapes and sizes. The traditional methods include physical, mechanical and chemical techniques. However, these methods are very expensive and some of them involve hazardous chemicals. Therefore, the development of environmetal friendly methods and safe processes is required. Organisms such as fungi, bacteria, plants and algae can catalyze specific reactions as a part of green biosynthetic strategies. In this study, we tested the potential exploitation of the freshwater green algae *Chlamydomonas reinhardtii* for the synthesis of silver nanoparticles (Ag NPs). We demonstrated that *in vivo* cells mediate the synthesis of Ag NPs in a light-dependent manner. An integrated approach of biological and chemical analysis is being carried out to investigate the role of the light in the synthesis kinetic, size and shape of nanoparticles.

PC6

Synthesis and optical properties of sulfanyl porphyrazines possessing phthalimide substituents in the periphery.

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Porphyrazine macrocycles constitute a novel class of porphyrinoids consisting of four pyrrole rings linked together with *meso* nitrogen bridges. Porphyrazines can be modified in their periphery with various substituents or groups. They possess interesting physicochemical properties, including fluorescence and the ability to generate singlet oxygen. Porphyrazines are considered as agents for nanotechnology and biomedicine, especially as photosensitizers for photodynamic therapy of cancer and noncancerous diseases [1,2].

The presented study aimed to obtain novel mercaptomaleonitrile derivatives possessing phthalimide substituents. Newly synthesized compounds were subsequently subjected to Linstead macrocyclization reaction using magnesium 1-butanolate in 1-butanol what resulted in the formation of symmetrical magnesium porphyrazines. In the next step, magnesium porphyrazine was used in demetallation reaction (with trifluoroacetic acid) followed by remetallation reaction using zinc(II) acetate. Obtained compounds were carefully purified and characterized using UV-Vis, NMR spectroscopy (including two-dimensional NMR techniques) and mass spectrometry. Moreover, metal ion containing macrocycles were subjected to further photochemical and photophysical studies including the assessment of singlet oxygen generation efficiency. .

The optimal therapeutic window of the light range for *in vivo* treatment is considered to be 600-800 nm, due to tissue permeability and haemoglobin or cytochromes absorption in the low wavelength region. A large number of photosensitizers have been evaluated for their potential clinical use. Many of them absorb light below 600 nm, thus limiting their therapeutic use [3]. Obtained macrocyclic compounds absorb light over 600 nm, therefore may be considered as new promising photosensitizers for PDT therapy.

Acknowledgements

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PC7

Multi-Functional Membranes for Water Treatment and Desalination with Photo-induced Antifouling Properties: Optimization of the Fabrication Conditions

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Access to clean water continues to be the most urgent and pressing global issue. The increasing scarcity of fresh water sources and the global demand for water is expected to grow in the oncoming decades, whith the urgent need to develop alternative water supplies, including seawater desalination, reuse and recycling of wastewater. Membrane-based separations for water treatment and desalination are playing an increasingly important role to provide adequate water resources of desirable quality for a wide spectrum of designated applications. H2020-MSCA-IF-project 'Enhanced-MUMs' is a multidisciplinary project that aims at bringing high innovation in the forefront research area of water treatment and desalination. Enhanced-MUMs targets the development of advanced multifunctional and low-cost polymeric membranes for water treatment and desalination. The main innovation resides in the combination of enhanced structural properties (high porosity and reinforcement) for improved desalination characteristics and light-induced antifouling and antimicrobial activity based on the loading of photosensitizers in the polymeric membrane.
The current work represents optimization of the preparation conditions of cellulose acetate membranes by phase inversion technique using automatic film applicator and water as a coagulation medium. The prepared bare membranes have a well-defined anisotropic microscopic structure with a pure water flux range from 150 L/m2.h to 990 L/m2.h using different membrane thickness and under different applied pressures. Modified membranes have been prepared using salt coagulation bath as well as in-situ salt addition to the polymer solution during the membrane fabrication. It was found that the preparation conditions greatly affect the microscopic and surface characteristics. The project also aims to: improve the microscopic structure with regard to the pore size and structure and improve the mechanical properties of the modified membranes. In addition, the project aims also to study and control the fouling characteristics of the membranes by imparting photo-induced antimicrobial and antifouling effects.

H2020-MSCA-IF-fund, project number 800317 'Enhanced-MUMs', is gratefully acknowledged.

PC8

Mitochondria-targeted Porphyrinoids: A New class of Photosensitizer for One and Two-photon Targeted Photodynamic Therapy

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Targeted cancer therapies offer a promising strategy to overcome problems associated with conventional treatments [1]. In this context, photodynamic therapy (PDT) received special attention due to its selective and minimally invasive treatment procedures [2]. However, some photosensitizers accumulate in healthy tissues and cause adverse effects during PDT action. Several "active" and "passive" targeting strategies are employed to enhance the specificity and efficiency of PDT [3]. Herein, our project aims to develop a new-class of photosensitizers (free base porphyrins, metallated porphyrins and corroles) to target mitochondria of tumor cells and thus enhancing one- and two-photon phototoxicity. Furthermore, photosensitizers encapsulated in liposomal nanoformulations will be studied for tumor targeting. These novel mitochondria targeted photosensitizers can overcome the problem of hypoxia in PDT and result in high efficacy of the therapy.

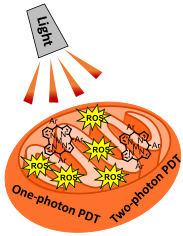

Fig. 1. General schematics proposing the role of new photosensitizers on targeted PDT.

The work is supported by the H2020-MSCA-IF-2017-798952 project "PROMPT".

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PC9

Boron-dipyrromethene (BODIPY) derivatives bearing N-alkyl phthalimide and amine substituents of potential application in the photoinactivation of bacteria

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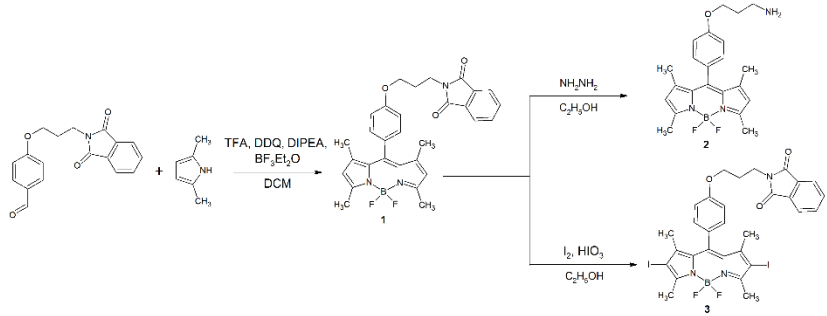
Boron dipyrromethene derivatives (BODIPY) form a new class of photosensitizers composed of two dipyrrole units connected with methine bridge, forming a tricyclic complex with boron as the central atom. BODIPY possess interesting optical properties, especially a highly efficient fluorescence. Therefore, they are often used as fluorescence probes for various biological molecules. On the other hand, it was demonstrated that the wavelength of light that is absorbed by BODIPY, as well as the ability to generate singlet oxygen can be easily modified by changing the substituents of the pyrrole rings. These properties make them promising candidates for the application in photodynamic therapy (PDT) [1,2].

Novel BODIPY derivatives bearing N-alkyl phthalimide (**1** or **3**) and amine (**2**) substituents were synthesized (Figure). Next, compounds were characterized using mass spectrometry, UV-vis spectrophotometry, and various NMR techniques. Subsequent studies aimed to evaluate their photochemical properties, including absorption and emission features, as well as the ability to generate singlet oxygen, which plays an essential role in PDT. It was found that the typical for BODIPY derivatives maximum absorption near 500 nm, can be bathochromically modified of about 30 nm, by the introduction of iodine atoms at positions 2 and 6 of BODIPY core. Moreover, the calculated value of singlet oxygen quantum yield increases drastically for an iodinated analogue **3**. Preliminary *in vitro* photodynamic antimicrobial activity studies were performed on Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* strains. It was found that all three BODIPY derivatives showed very high photoinactivation activity against *S. aureus*, but only analogue **2** with amine group revealed a significant effect on *E. coli.*



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This work was supported by the Polish National Science Centre under grant no. 2016/21/D/NZ7/01540

PC10

In vitro photodynamic antimicrobial activity of novel boron-dipyrromethene derivatives with aliphatic tertiary and quaternary amino substituents.

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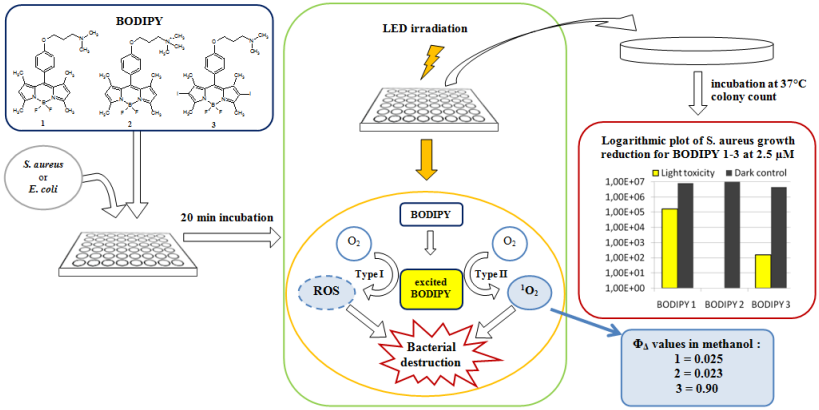
Traditional antimicrobial approaches are getting more and more often inefficient for the inactivation of pathogenic microorganisms. Many infectious diseases may become incurable due to growing antimicrobial resistance. Photodynamic antimicrobial chemotherapy (PACT) seems to be a very promising modality for the treatment of various superficial infections, including those induced by multi-resistant bacteria. PACT involves the use of a photosensitizer (PSs) and light of appropriate wavelength to induce oxidative stress which leads to eradication of bacterial strains [1]. Boron dipyrromethene (BODIPY) derivatives are organic chromophores possessing several attractive physicochemical properties that can be easily modified by structural changes. Bromine or iodine substitution in BODIPYs core drastically increases the generation of singlet oxygen, making them promising candidates for PACT. In addition, BODIPYs with positive charge shows optimal activity on both gram-positive and gram-negative bacteria [2,3].

A series of novel BODIPY derivatives were synthesized and characterized using mass spectrometry, UV-vis spectrophotometry, and various NMR techniques. Photochemical studies including absorption and emission properties, as well as the ability to singlet oxygen generation (Φ_{Δ}), were assessed. It was found that the introduction of iodine atoms to the BODIPY core caused an about 30 nm bathochromic shift of absorption band and also a much higher ability to generate singlet oxygen. In vitro photodynamic antimicrobial activity studies were performed on Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* strains. Tested compounds revealed high photodynamic activity against *S. aureus*. Moreover, **2** and **3** showed moderate activity against *E. coli*.



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Light-induced therapies (infections & dermatology)

IC7

Phototherapy in the Age of Biologics

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The prevalence of narrow-band ultraviolet B (NB-UVB) use in Europe for moderate and severe psoriasis is unknown, because national registries for psoriasis do not monitor this treatment. The aim of our study was to quantify the use of phototherapy, biologics or conventional treatments in psoriasis, in a setting where European Medicines Agency (EMA) eligibility criteria for biologics were strictly applied, and phototherapy was included among first-line treatments. In order to reach the target we followed a cohort of 1,090 patients who were referred to the only center entitled to prescribe biologics and phototherapy during a 5-year period. The cumulative number of treatment cycles was: 1,047 with NB-UVB phototherapy, 650 with systemic treatments and 239 with biologics; 754 patients received at least 1 course of NB-UVB phototherapy, 422 at least 1 course with a systemic treatment and 137 with a biologic; 595 patients were treated only with phototherapy. In conclusion the regular use of NB-UVB as first-line treatment for moderate and severe psoriasis and adherence to the EMA eligibility criteria for biologics led to a relatively restricted use of biologics.

OC15

Non-invasive evaluation of therapeutic response of multiple actinic keratosis of face and scalp treated with field cancerization treatments

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Background: To date there are no study directed to the high frequency ultrasound study of AKs response to conventional treatments.
Objective: To assess and compare clinical, dermoscopical and high frequency ultrasound features in multiple AKs of face and scalp and skin field of cancerization after field-directed treatments.
Matherial and Methods: We randomized 90 patients with Olsen II AKs of the face and scalp to MAL-Photodynamic therapy (MAL-PDT), ingenol mebutate 0.015% gel (IngMeb) and diclofenac 3% gel (DHA). At baseline and 3 months after treatment, clinical, dermoscopical and high frequency ultrasound features were assessed.
Results: MAL-PDT induced a higher AKs area and modified-AKASI score reduction. It induced a higher reduction of the SLEB thickness and an improvement of dermic and SLEB echogenicity.
Conclusions: MAL-PDT was the most effective treatment and ultrasound confirmed its anti-elastotic effect on SLEB. Increased dermal echogenicity after MAL-PDT was confirmed dermocopically by a reduction of lesional vascularization.

OC16

Antimicrobial photodynamic activity of RLP068/Cl in cutaneous infections: a pilot investigation

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The growing bacterial resistance against to conventional therapy is a rising public health and has renewed the search for alternative treatment modalities. Antimicrobial photodynamic therapy (APDT) uses a photosensitizer that targets bacterial cells following exposure to visible light and represents a suitable non-antibiotic broad-spectrum antimicrobial treatment against multidrug-resistant micro-organisms. A novel photosensitizer, called RLP068/Cl, is a tetracationic Zn(II)phthalocyanine derivative that have demonstrated a good efficacy against surgical wound infections induced by a methicillin-resistant strain of Staphylococcus aureus (MRSA) in mouse models and also against prosthetic joint infections-associated biofilms induced by Pseudomonas aeruginosa. We will present our preliminary findings in the treatment of some in vivo cutaneous infections (folliculitis, hidradenitis suppurativa, bacterial infection of lower limb ulcers, intertrigo), that has the potential to make APDT with RLP068/Cl an interesting therapeutic option in the landscape of the future treatment of skin infections.

Nanotechnology-mediated PDT

OC17

Keratin nanoparticles co-delivering Docetaxel and Chlorin e6 promote synergic interaction between chemo- and photo-dynamic therapies

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The combination of chemotherapy and photodynamic therapy (PDT) is considered a valuable strategy for increasing therapeutic response of cancer treatment, and the re-formulation of clinically approved pharmaceuticals in biocompatible nanoparticles (NPs) is particularly appealing for the possibility of co-loading, in a single delivery vehicle, drugs exerting cytotoxicity by different mechanisms, with the aim to synergistically kill cancer cells [1] [2]. In this overview, a novel keratin-based nanoformulation for the co-delivery of the antimitotic Docetaxel (DTX) and the photosensitizer Chlorin e6 (Ce6) was synthetized by us, completely in water solution and without the use of organic solvents and aggregatin agents. In fact, the drug-induced aggregation method [3] allowed the formation of monodisperse NPs (DTX/Ce6-KNPs) with an average diameter of 133 nm and loaded with a drug ratio of 1:1.8 of Ce6 *vs* DTX. Ce6, even if covalently linked to the keratin backbone in DTX/Ce6-KNPs, retained the ability to produce ROS and synglet oxygen upon light activation, while DTX release in PBS/EtOH displayed a monophasic release trend, which includes an initial burst during the first 5 h, followed by a more sustained release in the following 24 h of observation up to approximately 90% at 48 h.

The efficacy of DTX/Ce6-KNPs toward cancer cells was investigated *in vitro* in monolayers as well as in multicellular tumor spheroids of DTX-sensitive HeLa (HeLa-P) and DTX-resistant HeLa (HeLa-R) cells. In monolayers, the cytotoxic effects of DTX/Ce6-KNPs toward HeLa-P cells were comparable with those induced by free DTX + Ce6, while in HeLa-R cells the drug co-loading in KNPs resulted in a synergic interaction between chemotherapy and PDT, as calculated by applying the Chou and Talalay method [4]. Moreover, as respect to monotherapies, DTX/Ce6-KNPs induced stronger cytotoxicity to both HeLa-P and HeLa-R multicellular spheroids and reduced their volumes up to 50%, even if PS localization remained confined to the outer rims of cells of the spheroids. Overall, our results suggest that KNPs are very promising systems for the co-delivery of chemotherapeutics and PSs, favoring synergic interactions between PDT and chemotherapy.

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OC18

Mesenchymal stem cells as drug delivery system of dual loaed nanoparticles: a promising approach for osteosarcoma treatment.

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Clinical efficacy of many anticancer drugs is limited by the development of drug resistance and systemic drug toxicity, resulting in a low patients' survival rate. Photodynamic therapy (PDT) might represent an effective approach to overcome these drawbacks by killing tumor cells and circumventing chemoresistance [1]. On the other hand, nanotechnology-based drug delivery systems (DDS) can improve antineoplastic agents' efficacy, pharmacokinetic profiles, and selectivity to the target.

In the past decade, the use of human Mesenchymal Stem cells (hMSC) as cell-based drug delivery vectors to treat cancer, has significantly developed [2]. hMSC display the capacity to internalize and retain drugs and nanoformulations [3], and they can infiltrate the tumor stroma via cytokines-driven clues released from the tumor [4]. Osteosarcoma (OS) is the most common bone tumor diagnosed in children and adolescents. The development of chemoresistance and lung metastasis limit the success rate to a worldwide 70% 5-years patients survival.

The aim of this study is to engineer hMSC carrying DDS (hMSC-DDS) for OS treatment, using a combination of PDT (Chlorin e6 as photosensitizer) and chemotherapy (Paclitaxel-PTX, as antineoplastic) and test its efficacy in co-culture models.

DDS were prepared from high molecular weight and hydrosoluble keratin functionalized with Ce6 and loaded with PTX to obtain PTX-Ce6@ker nanoparticles (KNPs) [5]. Mesenchymal stromal cells are isolated from bone marrow of non oncologic patients who underwent elective surgery after informed consent. The cells are expanded and characterized in terms of proliferation, immunophenoty, differentiation and ability to internalize and retain the nanoformulations.Our results show that hMSC efficiently internalize KNPs after 24 h and retain them intracellularly for several days in culture. Their migratory capacity is partially reduced by PTX internalization, and in the 3D model, the cell death induced by PTX-cell cycle blockage and light irradiation is 95%.

Taken together, our data corroborate the use of MSCs-DDS as a high effective treatment in a 3D tumor model, and set the basis for the preclinical translation. If proved to be effective in vivo, this approach could revolutionise OS pharmacological treatment in affected patients.

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IC8

Magnetic Hyperthermia – Benefits and Drawbacks to the use of Physically-Triggered Iron Oxide Nanoparticles in the Clinic for Cancer

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Since 2011, Trinity College Dublin has been actively involved in European-wide projects involving the use of iron oxide nanoparticles to treat cancer through magnetic hyperthermia. In this treatment regime, magnetic nanoparticles are injected intratumourally into a patient, which is then followed by an exposure to an external magnetic field. The superparamagnetic properties of these nanoparticles enable them to heat up and induce apoptosis in the tumour, all while enhancing the effects of conventional therapies such as chemotherapy and radiation. Based on the early successful work of the Horizon 2020-funded MultiFun project, lead nanoparticles were identified and tested for efficacy in vivo in breast cancer and pancreatic cancer models. This led to the follow-on project – NoCanTher – which takes the knowledge and skills developed from this early work and aims to achieve clinical approval of an iron oxide nanoparticle to treat pancreatic cancer through this physically-triggered thermal therapy. This talk will describe the benefits of this therapy with reference to the successes of MultiFun and NoCanTher, and also highlight the potential drawbacks describing major translational issues such as endotoxin contamination and immunotoxicology.

Miscellaneous

OC19

Constructing hybrid photosynthetic artificial cells transducing light energy in ATP molecules

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Most of complex metabolic reactions in living cells involve different intracellular organelles with different biological functions. The idea to mimic living organisms is on the basis of the construction of the artificial cell.[1-3] The most challenging aspect is the control and the regulation of the biochemical reactions that involve complex membrane protein machinery.
Looking at the photosynthetic bacterium *Rhodobacter sphaeroides* as model organism, here we present a possible way to build a hybrid, artificial and natural, multicompartment system capable to transduce light energy in chemical energy stored in ATP molecules. This system consists of extracted nanometric bacterial vesicles, containing the entire photosynthetic apparatus, i.e. chromatophores[4], used as organelles when entrapped in a micrometric artificial cell-mimicking compartment, a giant lipid vesicle (GV). The photosynthetic apparatus consists of light harvesting complexes LH-I and LH-II, reaction center complexes (RC), coenzyme Q:cytochrome c – oxidoreductase (bc1) and ATP synthase complexes. The peculiarity of this system is its photo-inducibility: continuous infra-red light can trigger cyclic redox reactions producing a proton gradient across the membrane. This proton motive force is afterwards exploited, in presence of ADP and Pi molecules, by the ATP synthase to produce ATP molecules in the external environment (Figure 1). In this contribution we present an optimized chromatophore extraction procedure that brings to a sample of bacterial vesicles with desired orientation and retained photoactivity quantifying, by chemiluminescence assay, ATP production triggered by infra-red light.
Afterwards, we encapsulated these photosynthetic organelles in giant lipid vesicles obtaining hybrid photosynthetic artificial cells. After the encapsulation we verified that in absence of ADP molecules, the photosynthetic organelles were able, under continuous illumination, to induce an alkalinisation of the giant vesicle water core monitored with a pH-sensitive fluorescent dye, i.e. pyranine. The photosynthetically produced ATP within giant vesicles could be the fuel for sustaining simplified metabolic pathways for the synthesis of macromolecules such as proteins that can confer specific tasks to these artificial protocells.

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OC20

Crosstalk between ROS-Kras-Nrf2 axis and NF-kB/Snail/RKIP circuitry and its implications in PDT treatment.

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Pancreatic cancer cells are characterized by a high metabolic rate which correlates whit the increased production of reactive oxygen species (ROS). As high intracellular level of ROS is toxic and oxidize lipids, proteins and DNA, cancer cells are able to prevent ROS accumulation by upregulating antioxidant systems.
We found that the cationic porphyrin TMPyP4, once irradiated with white light, generates ROS producing an upregulation of KRAS and Nrf2, which is a transcriptor factor that behaves as the master regulator of redox homeostasis [1]. Moreover, we found that the ectopic expression of mutant KRAS G12D or G12V results in the upregulation of Nrf2 [2], suggesting that the oncogenic KRAS controls the redox homeostasis through Nrf2 expression. Therefore, we also found that an increase of ROS upregulates KRAS, and taken togheter these results show that in pancreatic cancer cells the redox balance is controlled by an axis composed by ROS, Kras and Nrf2. Our data suggest that this axis also affects the survival apoptosis pathways involving the NF-kB/Snail/RKIP circuitry. We demonstrated that low ROS levels, obtained when Nrf2 is activated by Kras, results in the upreglation of prosurvival Snail and downregulation of proapoptotic RKIP, favouring cell proliferation. By contrast, when Nrf2 is inhibited, the higher ROS levels generate a gene expression pattern that favours apoptosis by the upregulation of proapoptotic RKIP and downregulation of prosurvival Snail. These results can be useful to design an efficient photodynamic treatment starting from the observation that cancer cells can be better sensitized to photodynamic therapy if they are co-treated with a photosensitizer and an inhibitor of Nrf2. In other words, the inhibition of Nrf2 can increase the efficiency of PDT.

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OC21

a-ARM: Automatic Rhodopsin Modeling with Chromophore Cavity Generation, Ionization State Selection, and External Counterion Placement

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The automatic rhodopsin modeling (ARM) protocol[1] has recently been proposed as a tool for the fast, parallel and congruous generation of basic hybrid quantum mechanics/molecular mechanics (QM/MM) models of wild-type and mutant rhodopsins. However, in its present version, the input preparation requires a few hours long user's manipulation of the template protein structure which also impairs the reproducibility of the generated models. This limitation, which makes the model building semiautomatic rather than fully automatic, comprises four tasks: the definition of the retinal chromophore cavity, the assignment of the protonation states of the ionizable residues, the neutralization of the protein with external counterions, and finally the congruous generation of single or multiple mutations. In this talk, we will show that the automation of the original ARM protocol can be extended to a level suitable for performing the above tasks without user's manipulation and with an input preparation time of minutes. The new protocol, called *a*-ARM,[2] delivers fully reproducible (*i.e.*, user independent) rhodopsin QM/MM models as well as an improved model quality. More specifically, we show that the trend in vertical excitation energies observed for a set of 25 wild-type and 14 mutant rhodopsins, is predicted by the new protocol better than when using the original. Such an agreement is reflected by a trend deviation estimated as 0.7 ± 0.5 kcal mol^{-1} (0.03 ± 0.02 eV) and a mean absolute error of 1.0 kcal mol^{-1}(0.04 eV). Moreover, *a*-ARM is implemented in the web interface WEB-ARM[3] and it is available for the scientific community.

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OC22

Fluorescent Enhacement of a Microbial Rhodopsin via Electronic Reprogramming.

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In recent years, world economy and thecnologycal advancement have been transformed by Genomics, which allows us to study, design and build biologically relevant molecules. The engeneering of microbial rhodopsin proteins with enhacement fluorescence is of great importance in the expanding field of Optogenetics [1]. In the present combined experimental and theoretical study, we report the discovery of two mutants (W76S/Y179F and L83Q) of a light sensor from the fresh water eubacterium *Anabaena* with opposite fluorescence behavior [2]. In fact, while W76S/Y179F display a nearly 10-fold increase in red-light emission with respect to the wild-type protein, which exhibits a dim fluorescence, L83Q is not emissive. Thus, the W76S/Y179F, L83Q pair offers an unprecedent opportunity for the investigation of fluorescence enhacement in microbial rhodopsins, which is pursued by combining transient absorption spectroscopy [3] and multi-configurational quantum chemistry [4]. The results of such an investigation point to an isomerization-bloking electronic effect as the direct effect cause of instantaneous (subpicosecond) fluorescence enhacement. Accodingly, ASR W76S/Y179F or its variants may lead to promising voltage reporters for observing either the change in fluorescence intensity at a specific wavelength or the change in the emission wavelength (λ^{f}_{max}) as a function of time.

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