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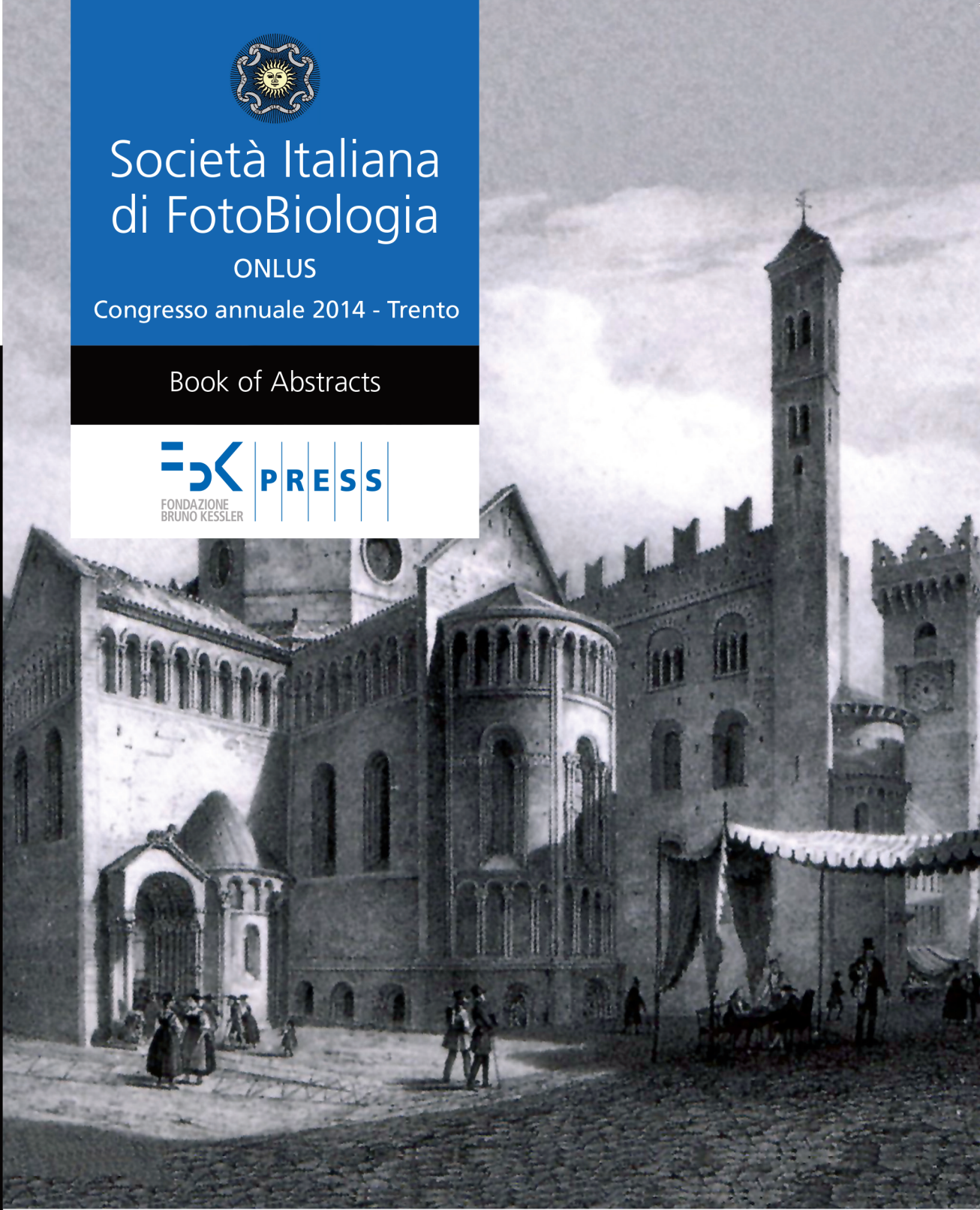

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Congresso Annuale 2014

Fondazione Bruno Kessler, Trento 11-13 giugno 2014

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FBK - Editoria: n. 04 / 05-2014

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PROGRAM

Mercoledì 11 giugno 2014

13:00-14:30	*Registrazione*
14:30-14:45	*Apertura del Congresso:* **Giorgia Miolo**, *Presidente SIFB*
	SESSIONE TECNICHE E METODI PER LA FOTOBIOLOGIA
	Chairs: **Ranieri Bizzarri (Pisa) – Giovanni Checcucci (Pisa)**
14:45-15:15	**IL1 – *Kevin Braeckmans (Ghent, B)*** *[invited lecture]* *Studying nanomedicine biobarriers by advanced fluorescence microscopy methods*
15:15-15:30	**OC01** – Greta VARCHI (BO): Polymeric core-shell nanoparticles: Targeted photo- and sono-dynamic therapy for cancer
15:30-15:45	**OC02** – Stefania ABBRUZZETTI (PR): A self-assembled nanostructured material for photodynamic therapy of tumors with potential for superresolution microscopy
15:45-16:15	*Coffee break*
16:15-16:45	**IL2 – *Roberto Bassi (Verona)*** *[invited lecture]* *Metabolic feed-back and photoreceptors control photoprotection mechanisms and photosynthetic energy conversion efficiency*
16:45-17:00	**OC03** – Andrea MENEGHESSO (PD): Light use optimization of microalgae for biofuel production
17:00-17:15	**OC04** – Leon J. JUAREZ-HERNANDEZ (TN): Channelrhodopsins as optogenetic tools to study hybrid neuronal-like architectures
17:15-17:30	**OC05** – Anna FASOLI (FE): Modulation of cone photoresponse by whole-cell delivery of zGCAP3 and its monoclonal antibody
17:30-17:45	**OC06** – Roberta RAGNI (BA): Organic conjugated oligomers: A versatile class of molecules for photobiological systems

Giovedì 12 Giugno 2014

SESSIONE MATERIALI PER LA FOTOBIOLOGIA

Chair: **Massimo Trotta (Bari)**

9:00-9:30	**IL3 – *Salvatore Sortino (Catania)*** *[invited lecture]* *Photoactivated nanoassemblies with multiple imaging and therapeutic modalities*
9:30-9:45	**OC07** – Roberta TATTI (TN): Functionalization of SiC nanowires by supersonic molecular beams for photodynamic therapy
9:45-10:00	**OC08** – Bogdan PARAKHONSKIY (TN): Vaterite particles as platform for photodynamic therapy
10:00-10:15	**OC09** – Francesco MILANO (BA) – The binding of quinone to the photosynthetic reaction centers
10:15-10:45	*Coffee break*
10:45-11:15	**IL4 – *David Stoppa (Trento)*** *[invited lecture]* *Single-photon image sensors for biomedical applications*
11:15-11:30	**OC10** – Laura PASQUARDINI (TN): Design of optical sensors for blood protein recognition
11:30-11:45	**OC11** – Enrico CARUSO (VA): New diarylporphyrins(di- and mono-cationic) as antitumor and antibacterial photosensitizers

SESSIONE FOTODERMATOLOGIA

Chair: **Marina Venturini (Brescia)**

11:45-12:15	**IL5 - *Piergiacomo Calzavara-Pinton (Brescia)*** *[invited lecture]* *Photodermatology and photobiology: Possible contact points*
12:15-12:30	**OC12** – Maria Teresa ROSSI (BS): Cutaneous distribution of plasmacytoid dendritic cells in Lupus Erythematosus, cutaneous Lupus Erythematosus and polymorphic light eruption
12:30-12:45	**OC13** – Marina VENTURINI (BS): Reflectance confocal microscopy for *in vivo* evaluation of basal cell carcinoma's surgical margin

12:45-13:00	**OC14** – Mariachiara ARISI (BS): Hydroa Vacciniforme: A rare photodermatosis EBV-associated. Report of two clinical cases
13:00-13:15	**OC15** – Alessandra SEMENZATO (PD): New nanovehicles for 5-ALA PDT treatments: Formulation development and stability studies
13:15-15:00	*Lunch*
	SESSIONE PDT: ASPETTI MOLECOLARI Chair: **Valentina Rapozzi (Udine)**
15:00-15:30	**IL6 – *Luciana Dini (Lecce)*** *[invited lecture]* *Immunogenic cell death in Rose Bengal Acetate-PDT*
15:30-15:45	**OC16** – Emilia DELLA PIETRA (UD): Use of a photosensitizer-NO conjugate to improve photodynamic therapy in prostate cancer cells
15:45-16:00	**OC17** – Francesca MORET (PD): Cationic antimicrobial peptides are efficient carriers for the delivery of porphyrins to cancer cells
16:00-16:30	*Coffee break*
16:30-17:00	**YKL1 – *Federica Barra (Napoli)*** *[young keynote lecture]* *5-ALA/PDT induced damage in cells: Unravelling the roles of p53 and ABCG2 transporter*
	SESSIONE PDT ANTIMICROBICA Chair: **Enrico Caruso (Varese)**
17:00-17:30	**IL7 – *Viviana Orlandi (Varese)*** *[invited lecture]* *Photodynamic treatment: How do prokaryotes respond?*
17:30-17:45	**OC18** – Enrico CARUSO (VA): Synthesis of a series of BODIPYs, photodynamic activity and QSAR model
17:45-18:00	**OC19** – Pietro DEL CANALE (PR): Proteins as biocompatible nanocarriers of hydrophobic photodynamic drugs: The complex of hypericin with β-lactoglobulin
18:00-19:00	*Assemblea dei Soci SIFB*
20:30	*Cena Sociale*

Venerdì 13 Giugno 2014

SESSIONE FOTOTERMICA E FOTOACUSTICA

Chair: **Giovanni Romano (Firenze) – Roberto Pini (Firenze)**

9:00-9:30	**IL8 – *Roberta Ramponi (Milano)*** *[invited lecture]* *Single-cell manipulation in femtosecond-laser-fabricated optofluidic devices*
9:30-9:45	**OC20** – Giovanni ROMANO (FI): Innovative phototherapy for the treatment of *Helicobacter pylori* infection
9:45-10:00	**OC21** – Alessio GNERUCCI (FI): Optical hyperthermia of tumor cells via targeted gold nanorods
10:00-10:15	***Sponsor's Technical Communication*** *– HAMAMATSU (Laura CONFALONIERI)*
10:15-10:45	*Coffee break*

SESSIONE FOTOTOSSICITÀ E FOTOSTABILITÀ

Chair: **Giorgia Miolo (Padova)**

10:45-11:15	**IL9 - *Paola Calza (Torino)*** *[invited lecture]* *Transformation processes of drugs occurring in different matrices*
11:15-11:30	**OC22** – Giorgia MIOLO (PD): Photostability of morphine and 6-MAM exposed to controlled UVB/UVA irradiation in water and methanol solution. Comparison with data in the solid state
11:30-11:45	**OC23** – Marianna TUCCI (PD): A study on photodegradation of methadone, EDDP and other drugs of abuse in hair exposed to controlled UVB radiation and comparison to data obtained in methanol solution
11:45-12:00	**OC24** – Albrecht HAASE (TN) Photodamage in two-photon optical tomography of human skin
12:00-12-15	**OC25** – Alessandra MAZZOLI (PG): Spectroscopic Investigation of the molecular interaction of new potential anticancer drugs with DNA and non-ionic micelles
12:15-12:30	*Saluti Finali e Chiusura del Congresso*
12:30-14:00	*Farewell Lunch*

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TECHNIQUES AND METHODS FOR PHOTOBIOLOGY

Studying Nanomedicine Biobarriers by Advanced Fluorescence Microscopy Methods

Kevin Braeckmans[1,2], Katrien Remaut[1], Koen Raemdonck[1], Jo Demeester[1], and Stefaan C. De Smedt[1]

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In the drug delivery field, interest goes to developing 'intelligent' nanoscopic particles that are capable of efficiently delivering biopharmaceuticals to target cells. These nanoparticle formulations should fulfill several requirements. Besides efficiently encapsulating the biopharmaceuticals, they also have to provide protection against degradation during the entire delivery process. Furthermore, the nanoparticles should not aggregate e.g. after intravenous injection. Nor should they release the therapeutic cargo while being suspended in the blood circulation or when traversing the extracellular space. Release of the biopharmaceuticals in many cases should only occur after being internalized in the target cells. Obtaining a better insight into the physicochemical and biophysical behaviour of the nanoparticles during the various phases of the delivery process is required to achieve efficient optimization of their structure and composition.

For more than 10 years, our group has been exploring the use of advanced fluorescence microscopy methods for this purpose. Detailed information on the mobility and potential binding of nanoparticles in extracellular tissues can be obtained using fluorescence recovery after photobleaching (FRAP) or fluorescence Single Particle Tracking (fSPT). These techniques were for instance used to examine the mobility of nanoparticles in lung sputum of cystic fibrosis patients and vitreous humour. We have used fluorescence correlation spectroscopy (FCS) to study the association and dissociation of nucleic acids from nanomedicine formulations in biological media, such as sera, cell lysates and cells. Complementary to that, fSPT has proven to be a unique tool to accurately measure nanoparticle aggregation and concentration in biological fluids. Finally, using fSPT and live cell microscopy we succeeded in unraveling the intracellular trafficking of nanoparticles in great detail.

By providing a better insight into the stability and transport of nanoparticles during the various phases of the delivery process through the use of advanced microscopy techniques, it is our aim to enable a more efficient and rational development of improved carrier materials for the delivery of nucleic acids.

Polymeric Core-shell Nanoparticles: Targeted Photo- and Sono-Dynamic Therapy for Cancer

Greta Varchi[1], Serena Duchi[2,3], Loredana Serpe[4], Roberto Canaparo[4], Giovanna Sotgiu[1], Andrea Guerrini[1], Marco Ballestri[1], Davide Donati[2,3], and Enrico Lucarelli[2]

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Although progress in basic research has led to the design of new generations of anticancer targeted drugs with some notable achievements further progress in cancer treatment may be accomplished through other existing, but still under-appreciated, therapeutic approaches. Among these, photo- and sono-dynamic therapies take advantage respectively from the use of light and non-thermal ultrasound to activate chemical compounds known as sensitizers. The activated sensitizer agent is then able to kill cancer cells through the generation of highly reactive products, such as reactive oxygen species (ROS), through apoptotic and/or necrotic mechanism. The great advantage of these techniques relies on their low systemic toxicity, the possibility of highly controlled non-invasive treatments/practices and the non-occurrence of drug resistance even after repeated treatment. Within this framework we developed and studied *in vitro* and *in vivo* the use of biocompatible, polymeric core-shell nanoaparticles (NPs) as multi-functionalized carriers of properly selected sensitizers for tumor treatment. In a fascinating study, we used the sensitizer loaded NPs in combination with mesenchymal stem cells as carriers of the loaded NPs. In the study we aimed to exploit the natural tropism of MSC towards inflammated sites to improve the targetability of our system. Preliminary *in vitro* and *in vivo* results confirmed the antiproliferative effect of our system, both under light irradiation and shock waves stimuli, in different solid tumors.

A Self-Assembled Nanostructured Material for Photodynamic Therapy of Tumors with Potential for Superresolution Microscopy

Pietro Delcanale[1], Stefania Abbruzzetti[1], Massimiliano Tognolini[2], Paolo Bianchini[3], Francesca Pennacchietti[3], Alberto Diaspro[3], Luigi Cavanna[4], and Cristiano Viappiani[1]

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Hypericin (Hyp) is a pigment extracted from the plant *Hypericum perforatum*. Hyp is present in dark glandular structures in the petals and it has been used as an antiviral, anti-bacterial and antifungal agent, and for the treatment of tumors. Singlet oxygen is photosensitized with high efficiency by monomeric Hyp. Hyp is insoluble in water, thus water soluble delivery agents are needed to overcome Hyp aggregation. We have recently shown that Hyp is accomodated in the hydrophobic distal cavity of apomyoglobin (ApoMb), thanks to the close similarity in size and shape with the physiological haem cofactor. The self assembled ApoMb-Hyp construct can efficiently transport the monomeric photosensitizer to target cells. Photo-toxicity tests on PC-3 cancer cells incubated with ApoMb-Hyp (upon exposure to green or blue light) show high toxicity in the light, almost equal to that of Hyp alone (survival of cells around the 5 %), but a lower toxicity in the dark. Finally, the inherent fluorescence emission of ApoMb-Hyp construct has been exploited to collect STimulated Emission Depletion (STED) Microscopy images of cancer cells loaded with the ApoMb-Hyp construct, demonstrating for the first time the potential of Hyp as a probe for superresolution microscopy.

Metabolic Feed-back and Photoreceptors Control Photoprotection Mechanisms and Photosynthetic Energy Conversion Efficiency

Alberta Pinnola[1], Stefano Cazzaniga[1], Luca Dall'Osto[1], and Roberto Bassi[3]

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All oxygenic photosynthetic organisms live on light energy and yet are inhibited by excess light because of reactive oxygen species produced by the reaction of chlorophyll triplet states with molecular oxygen they produce by oxydizing water. Because of the daily chage in light intensity and further sudden variations in photon fluence in natural environment, excess light exposure is a continuous experience for plants and algae thus leading to a limitation in photosynthetic growth and biomass accumulation. Photoprotective mechanisms include chloroplast avoidance and NPQ (non-Photochemical Quenching). We evaluated their relative importance in regulating excitation pressure on Photosystem II by comparing photosensitivity of genotypes impaired in chloroplast avoidance response (phot2), synthesis of zeaxanthin (npq1) or the activation of NPQ (npq4). Suppression of avoidance response resulted in oxidative stress under excess light, while removing either zeaxanthin or PsbS had a milder effect. The double mutants phot2 npq1 and phot2 npq4 showed the highest sensitivity to photooxidative stress, implying additive effects. Under different intermittent light regimes, the relative importance of phot 2-mediated and PSBS/zeaxanthin mediated mechanisms changes with photoinhibition by short light pulses being counteracted by the latter while protection by longer light exposures being provided by the formers. Non-Photochemical quenching reactions decreases the growth rates of plants and algae by dissipating photons into heat. Engineering of the NPQ catalyzing components allows for increased photon conversion in biomass and biofuels in photobioreactors.

References:

Cazzaniga S., L. Dall'Osto, S-G Kong, M. Wada, R. Bassi (2013) Differential triggering by red vs. white light of chloroplast avoidance and excess energy dissipation in Arabidopsis thaliana allows for evaluation of their relative effect in photoprotection. The Plant Journal, 76(4): 568-79.

Pinnola A., L. Dall'Osto, C. Gerotto, T. Morosinotto, R. Bassi, A. Alboresi (2013). Enhancement of Non-Photochemical Quenching by Zeaxanthin involves its binding to the LHCSR protein in the moss *Physcomitrella patens*. The Plant Cell, 25(9): 3519-34.

Light Use Optimization of Microalgae for Biofuels Production

Andrea Meneghesso[1], Diana Simionato[1], Giovanni Finazzi[2], and Tomas Morosinotto[1]

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The problems linked to extensive exploitation of fossil fuels such as increasing costs due to feedstock scarcity and the increasing accumulation of CO_2 in the atmosphere suggest that drastic changes in our energy supply will be necessary in next decades. Research on new renewable energy sources is highly strategic and in this context the exploitation of microalgae is considered a promising alternative. Microalgae are photosynthetic unicellular organisms which can grow at a much faster rate than plants and have the ability to accumulate large amounts of lipids, up to 70% of total dry weight in species of the genus *Nannochloropsis*.

Solar light provides energy to support all metabolism of photosynthetic organisms but if absorbed in excess illumination may easily drive the production of reactive oxygen species and damage of the photosynthetic apparatus. Different species of microalgae evolved the ability of effectively responding to variations in light intensity and they maximize the light harvesting efficiency to support photosynthesis when solar radiation is limiting while dissipating any energy in excess. That capacity to acclimating to different light intensity makes these organisms valuable candidates for outdoor productions where illumination conditions are highly variable.

This work focuses on the seawater microalgae *Nannochloropsis gaditana*, a species of microalgae particularly promising for biodiesel production.

We've studied the combined effects of light intensity, nutrients and CO_2 concentrations on photosynthetic productivities. For all this conditions we have characterized the growth, the lipid productivities, the pigments content and the photosynthetic efficiency parameters.

The responses of *Nannochloropsis gaditana* to different conditions have been analyzed both assessing fast protection mechanisms such as the Non Photochemical Quenching (NPQ) as well as the long term acclimation to different light intensities. We have considered also other important photosynthetic efficiency parameters involved in the regulatory response of the photosynthetic apparatus such as the functional antenna size of the Photosystem II and the electron transport rate through Photosystem I.

Channelrhodopsins as Optogenetic Tools to Study Hybrid Neuronal-Like Architectures

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Channelrhodopsins, ChRs, are novel photoreceptors which act as directly light-gated ion channels: they become permeable to cations upon blue light stimulation and they appear the most versatile to be expressed in several cell model systems. Very recently a ChR-based technology, named optogenetics, has been developed to control and perturb cell physiology with a high spatio-temporal resolution. ChRs as optogenetic tools are genetically-encoded photoswitches able to trigger, control and modify specific cellular and/or cross-stalk activities. Targeted cell populations can be genetically sensitized via the heterologous expression of ChRs and upon 460-480 nm light stimulation: They are able to depolarize the cell membrane producing an excitation of the system. ChR proteins can be mutated and tailored with respect to absorption, substrate specificity, kinetics, and improved expression in host systems.

Our aim is to apply optogenetics to the study the interfacing of artificial memristive architectures with biosystems for novel approaches to information transfer and processing in bio-electronics. ChR-expressing cells could provide endogenous non-invasive trigger and control of excitability in cells interfacing the memristors. We started to select suitable cell models and to transfect them with recombinant viruses to express ChRs.

We used pGEMHE plasmids containing the genes of Channelrhodopsin-2 wild type or its mutant in position 159 both fused to YFP, named respectively chop2-315YFP and ChR2(T159C). We successfully obtained remarkable transfections in HeLa, an immortal cell line derived from tumoral human cervical cells, and in undifferentiated NSC-34 cells, a mouse motoneuron-like hybrid cell line. We positively checked the transfection efficiency and pattern with confocal microscopy analysis by protocols used for GFP excitation at 488 nm. Next steps will face the selection of suitable cell lines and the recording of the electrophysiological signals produced by the hybrid architecture. Our goal is to demonstrate that ChRs could be suitable optogenetic tools for the functional analysis of hybrid systems mimicking neural activity and plasticity.

Granted by Provincia Autonoma di Trento "Great Projects 2012 – MaDEleNA".

Modulation of Cone Photoresponse by Whole-Cell Delivery of zGCAP3 and its Monoclonal Antibody

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The physiological function of guanylate cyclase-activating protein 3 (zGCAP3) was investigated in zebrafish green-sensitive cones by recording the effect on the photoresponse by cytosol injection of exogenous zGCAP3 (to simulate protein over-expression), and its monoclonal antibody (to simulate protein knock-down). These proteins were delivered via whole-cell by an internal perfusion system coupled to a pressure-polished patch pipette. Whole-cell recordings performed for the first time on these cones, had stable light sensitivity, dark current amplitude, response kinetics and light adaptation. The rising phase of the response to saturating flashes was particularly fast (current fell to 0 within ~12 ms), while the recovery phase of the response to sub-saturating flashes was monotonic or biphasic suggesting, among other possibilities, the existence of two physiologically distinct cones having similar spectral sensitivity. Injection of anti-zGCAP3 produced current fall to zero level in ~5 min of antibody perfusion, and progressively slowing down kinetics of responses delivered on decaying current; however, control antibody gave similar results as anti-zGCAP3. Purified zGCAP3 did not alter the photoresponse, indicating that the target GC was already saturated with endogenous zGCAP.

Organic Conjugated Oligomers: a Versatile Class of Molecules for Photobiological Systems

Alessandra Operamolla[1], Roberta Ragni[1], Francesco Milano[2], Rocco Roberto Tangorra[1], Omar Hassan Omar[3], Angela Agostiano[1,2], Massimo Trotta[2], and Gianluca M. Farinola[1]

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Integrate photoactive enzymes or proteins with organic molecules tailored to enforce specific functions, represents a very intriguing research topic opening the way to the development of new hybrid biomimetic materials having functional properties going beyond those of natural systems. The versatility of organic and organometallic synthetic methodologies al.lows the preparation of a wide variety of molecular compounds with different basic chemical structure and the insertion of proper reactive groups which enable their covalent linking to specific sites of proteins. Indeed, proper selection of chemical structure is crucial to finely tune the photophysical and spectroscopic properties (e.g. absorbance, fluorescence or phosphorescence) of these molecules, as well as to control their molecular shape and flexibility avoiding sterical hindrance that can prevent their covalent linking to biomolecules.

The potential application of the organometallic synthetic methodologies applied to class of aryleneethynylenes compounds will be illustrated along with some perspectives in the fields of photobiology, photocatalysis and biological photovoltaics.

MATERIALS FOR PHOTOBIOLOGY

Photoactivated Nanoassemblies with Multiple Imaging and Therapeutic Modalities

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The achievement of molecular assemblies able to release multiple therapeutic species in a controlled fashion is a major challenge in the burgeoning field of nanomedicine.[1] Light is a powerful tool for the introduction of bio-active agents in a cellular environment, mimicking an "*optical syringe*" with an exquisite control of three main factors, site, timing and dosage, determining for the therapeutic outcome.[2] Moreover light-triggering is biofriendly and offers the additional advantages of not affecting important physiological parameters such as temperature, pH and ionic strength. In this context, the use of photoactive compounds having intrinsic fluorescence properties or their integration with suitable fluorogenic units is a fundamental requisite for an imaging-guided phototherapy. This allows indeed the visualization of the phototherapeutic agent in cells through fluorescence techniques and can provide a highly localized "burst" precisely at the desired sites. In addition, nanoassemblies co-delivering therapeutic and imaging functions may be designed in order to quantify in real time the amount of the released active species by fluorescence techniques and/or to monitor the biomedical response after the release with nanoscaled precision.[3] In our laboratories we have been working on the design and fabrication of a number of nanoconstructs merging multiple phototherapeutic and imaging modalities. This contribution illustrates some of the most recent and representative examples including polymer nanoparticles, hydrogels and carbon quantum dots working under one and two-photon excitation. The rationale design and the potential relevance of these systems in biomedical research will be highlighted.

1 Komarova, N.L.; Boland, C.R. Cancer: Calculated Treatment. *Nature* **2013**, *499*, 291–292.

2 Sortino, S. *J. Mater. Chem*., **2012**, *22,* 301-318.

3 Swaminathan, S.; Garcia-Amorós, J.; Fraix, A.; Kandoth, N.; Sortino, S.; Raymo, F. M.; *Chem. Soc. Rev.,* **2014** in press.

Functionalization of SiC Nanowires by Supersonic Molecular Beams for Photodynamic Therapy

R. Tatti[1], L. Aversa[1], R. Verucchi[1], F. Fabbri[2], F. Rossi[2], G. Attolini[2], M. Bosi[2], G. Salviati, and S. Iannotta[2]

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Photodynamic Therapy (PDT) is a therapeutical approach in the cancer treatment, which consists in the activation of a photosensitizer (phorphyrins) with visible light in order to produce singlet oxygen, to exert a cytotoxic activity towards cancer cells. The use of visible light limits the PDT application only to shallow deseases, for this reason we propose a new "X-Ray induced PDT" approach, using nanohybrid systems as photosensitizer, consisting in Silicon Carbide (SiC) nanowires (NWs) functionalized with organic molecules (fluorinated porphyrins). Modification of the inorganic semiconductor surface with organic or bio-molecules represents the route to activate processes at the interface and can be achieved by functionalizing the SiC NWs with porphyrins, that showing a good match between the organic absorption Q band and the 3C-SiC near-band-edge-optical emission. In fact SiC NWs have interesting light emission properties [1] so it is promising the idea to couple this light emission with an organic absorber showing strong fluorescence properties, a viable route to increase the optical emission efficiency as well as to promote the anchoring of biological groups. We demonstrated the functionalization of SiO_x/SiC core shell NWs, grown by a carbothermal method, showing enhanced fluorescence, with fluorinated porphyrins H2TPP(F) by supersonic molecular beam deposition (SuMBD), an approach that can promote and activate chemical/physical processes at the interface by means of the organic precursor kinetics properties [2]. The H2TPP(F)/SiC-NWs system has been deeply investigated *in-situ* with surface photoelectron spectroscopy and ex-situ by Cathodoluminescence (CL) to clarify the growth kinetics at low coverages and the interface processes. Results concerning the core level (C1s, Si2p, N1s, F1s) analysis at different growth steps on planar oxidized surface and SiOx/SiC core shell NWs will be presented. The role of morphology of inorganic surfaces together with kinetic activation of H2TPP(F) molecules in molecular beams will be discussed.

1 F. Fabbri et al., Nanotechnology In Press (2014)

2 M. Nardi et al., Phys. Rev. B 79, 125404 (2009)

Vaterite Particles as Platform for Photodynamic Therapy

B. Parakhonskiy[1,3], Yu. Svenskaya[1,2], A. Haase[4], E. Lukiyants[5], D. Gorin[2], and R. Antolini[4]

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The photodynamic is the one of the promising technique for anticancer therapy and treatment. But a negative side effect of this therapy is caused by its insufficient selectivity of action: a high concentration of photosensitizer is required for cancer treatment at the tumor site, but causes incidental toxicity in healthy tissue and increases the cost of the treatment. Side effects and treatment costs could be strongly reduced by targeted delivery to the region of interest at a well-defined time. In our work we use drug photosensitizer "Photosens" (a mixture of sulfonated aluminum phthalocyanines AlPcSn, with n = 2, 3 or 4).

A possible delivery system is particles based on the vaterite phase of calcium carbonate ($CaCO_3$). Vaterite particles has a large porosity, large surface area, and can decompose fast under relatively mild conditions. Our previous studies described the possibility of synthesizing spherical, elliptic and star-like mono-dispersed vaterite particles in the size range from 400nm to 10. A controllable release mechanism based on a crystal phase transition has recently been demonstrated. A number of necessary tests like the availability, cell toxicity, cell uptake were demonstrate. Also, the proof of principle using these containers was demonstrated by the pH sensitivity of the system.

We investigated the encapsulation efficiency for the photosensitizer in micrometer- and sub-micrometer-sized carriers. Release mechanism dependent on the surrounding pH was studied, showing a fast degradation of the carriers in buffers below pH 7.

These results hold out the prospect of a novel drug delivery system. Cancer-sensitivity can be achieved due to the enhanced uptake and fast release in the low pH endocytic vesicles of viable cancer cells.

The Binding of Quinone to the Photosynthetic Reaction Centers

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Liposomes represent a versatile biomimetic environment for studying the interaction between integral membrane proteins and hydrophobic ligands. In this paper, the quinone binding to the Q_B-site of the photosynthetic reaction centers (RC) from *Rhodobacter sphaeroides* has been investigated in liposomes prepared with either the zwitterionic phosphatidylcholine (PC) or the negatively charged phosphatidylglycerol (PG) to highlight the role of the different phospholipid polar heads. Quinone binding (K_Q) and interquinone electron transfer (L_{AB}) equilibrium constants in the two type of liposomes were obtained by charge recombination reaction of Q_B-depleted RC in the presence of increasing amounts of ubiquinone-10 over the temperature interval 6 – 35 °C. The kinetic of the charge recombination reactions have been fitted by numerically solving the ordinary differential equations (ODE) set associated to a detailed kinetic scheme involving electron transfer reactions coupled with quinone release and uptake. By using the sole quinone release constants (both in neutral and charge separated state) as adjustable parameters, the entire set of traces at each temperature was accurately fitted. The temperature dependence of the quinone exchange rate at the Q_B-site was hence obtained, finding that the quinone exchange regime is always fast for PC while it switches from slow to fast in PG as temperature rises above 20°C. In this paper was introduced a new method for the evaluation of constant K_Q using the area underneath the charge recombination traces as indicator of the amount of quinone bound to Q_B-site.

Lipid environmental modulation of activity of photosynthetic membrane proteins. L. Catucci, et al. (2008) in **Advances In Planar Lipid Bilayers And Liposomes**, Vol. 8, pages 27-57, ISBN-13: 978-0-12-374341-1, Academic Press

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Single-Photon Image Sensors for Biomedical Applications

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The impressive advancements in CMOS technologies over the last few decades have resulted in image sensors being a ubiquitous part of everyday life. However, there are always new challenges keeping research alive in the field of solid-state image sensors, with an increasing demand for imaging systems able to provide extra-information with respect to the standard digital cameras output. Among them there is the continuous progress of Single-Photon Avalanche Diode (SPAD) fabricated using standard CMOS technologies, which allow adding more and more, processing features onto the same chip while the pixel dimensions are shrinking. This kind of sensors, capable of detecting single quanta of light and resolving the photons time-of-arrival on a sub-nanosecond time scale, can be exploited in life science research for real-time fluorescence lifetime imaging, positron emission tomography, and lab-on-chip detection. In this contribution, a summary of the operation principle behind SPAD-based imagers and an overview of the technological developments in this field will be given, providing a perspective toward future biomedical applications.

Design of Optical Sensors for Blood Protein Recognition

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The introduction of new compact systems for sensitive, fast and simplified analysis is currently playing a substantial role in the development of point-of-care solutions aimed at helping diagnosis. Here we report on the advantages of using DNA aptamers, short oligonucleotide sequences that bind non-nucleic acid targets with high affinity and specificity, to develop a functional layer for biosensing. Thrombin and vascular endothelial growth factor (VEGF) are utilized as targets of aptamers: thrombin belongs to the coagulation cascade and it is involved in many pathological diseases, like atherosclerosis and thromboembolic diseases. VEGF is an important regulator of angiogenesis and it promotes the migration and proliferation of endothelial cells and the formation of new blood vessels from pre-existing capillaries. Different aptamer based platforms are presented. A first system is based on fluorescence detection, combining the high-performances of a Single Photon Avalanche Diode (SPAD) to an amplification obtained with two antibodies. The whole system, contained in a portable box with pumps and microfluidics, is validated against the human thrombin protein. Another platform takes advantage of a chemiluminescence reaction. Custom SPAD detectors with low dark-count rate had been directly coupled with the aptamer biorecognition layer, avoiding the use of optical components in the detection path. In this way, the signal to be detected develops very near to the sensor surface, maximizing the light collection in a very simple way. Preliminary tests confirm a great increase in sensitivity. The last presented biosensor system is based on a label-free approach such as whispering gallery mode (WGM) resonators, which are receiving a growing interest as optical structures suitable for the realization of miniaturized sensors with high sensitivity. When properly excited, WGM microresonators are able to strongly confine light, along the equatorial plane of the microresonator. Here we present two different approaches for the realization of such microresonators: i) a spherical microcavity with a Q factor value of 10^7, and ii) a planar wedge vertically coupled microdisk with a Q factor value of 10^4.

New Diarylporphyrins(di- and mono-Cationic) as Antitumor and Antibacterial Photosensitizers

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Photodynamic therapy (PDT) is based on the systemic or topical administration of a non toxic compound, called photosensitizer (PS), followed by an incubation period. The treated area is then irradiated with light of a desired wavelength, to activated the PS. Upon activation, the PS switches from its ground state to an excited singlet state, initiating a chain of electronic transitions that results in the production of death-inducing reactive oxygen species (ROS), mainly singlet oxygen ($^1O_2$). This treatment can be used as an alternative to the most common tumor therapies or as antibacterial treatment (in this case it is called PACT). A small number of PSs have been approved for clinical PDT applications on different cancer types, but many more are undergoing clinical trials. For the most part, these new entries retain the cyclic tetrapyrrole structure typical of porphyrin and of derivatives (chlorins and phthalocyanines).

In this work it is reported the synthesis of two new diarylic symmetric porphyrins (dicationic), characterized by the presence of two different spaces between the pyridinium moieties and the tetrapyrrolic ring, and a new diaryl asymmetric porphyrin bearing a positive charge on one side and a lipophilic substituent on the other.

The structure of these molecules has been chosen in order to mimic the structure of the phospholipids and with the chance that the same molecule could be applicable in both the antitumor and antimicrobial field just modifying the experimental conditions (i.e. incubation and irradiation time).

The new molecules were tested *in vitro* on two tumor cell lines (SKOV3 and HCT116), under different experimental conditions of incubation and irradiation. Our standard conditions for the *in vitro* tumor cells treatment require 24h of incubation time and 2h of irradiation. In new experimental conditions the incubation time and the irradiation time were reduced to 1h to get conditions similar to those applied for the antibacterial treatment.

Besides photodynamic experiments were set up on two bacterial strains, a Gram-negative (*Escherichia coli*) and a Gram-positive (*Staphylococcus aureus*) providing an incubation time of 10 min and exposure to a light source for 1h.

These different protocols have been developed in order to identify the suitable experimental conditions to apply the same molecule both PDT and PACT.

PHOTODERMATOLOGY

Photodermatology and Photobiology: Possible Contact Points

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In a scientific society of photobiology, a dermatologist is looking for collaborations and knowledge for the development of:

1) ultraviolet light sources for phototherapy (UVB, PUVA, bath PUVA, UVA1) and provoking phototests (unmet needs: narrow emission spectrum, modularity of the emission unit to match the geometrical irregularities of the human body surface, high emission power, low electrical supply)
2) visible light sources for photodynamic therapy of skin cancer and laser (surgical, pigmentary, vascular) (unmet needs: shorter durations of the impulse, duration of the equipment).
3) cheap and affordable spectrophotometers and broad band photometers.
4) new photosensitizers for diagnostics (staining for *in vivo* confocal microscopy?) and photodynamic therapy of inflammatory, infectious and tumoral skin diseases. (Just a question: is photochemotherapy definitely dead?)
5) improvement of the knowledge of the molecular changes in human irradiated cell populations.
6) Oral and topical light filters and agents liable to repair the photodamages.

Cutaneous Distribution of Plasmacytoid Dendritic Cells in Lupus Erythematosus, Cutaneous Lupus Erythematosus and Polymorphic Light Eruption

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Polymorphic light eruption (PLE) is the most common autoimmune photodermatosis but its pathogenesis remains to be clarified. An autoimmune reaction to unknown photoinduced antigens seems to play a major role because PLE patients are resistant to UV-induced immunosuppression. The hypothesis of an autoimmune mechanism is supported also by the high prevalence of PLE in patients affected by lupus erythematosus (LE) and their first-degree relatives. It has been demonstrated that Interferon (IFN)-α and its dominant cellular source, the plasmacytoid dendritic cell (PDC), play a crucial role in the in the pathogenesis of LE skin lesions irrespectively of photosensitivity. PDC are recruited to skin lesions and interact with other leucocytes to generate auto-reactive clones of lymphocytes and peripheral tissue damage. The role of PDC in PLE has still to be clarified.

We therefore conducted a study in order to identify the presence and localization of PDC in 75 LE skin biopsies (57 CLE, 18 LE), 24 PLE biopsies and seven normal control skin. For PLE patients the biopsy was performed in the site of positive phototest reaction 24 hours after last irradiation. Four micron tissue sections were used for immuno-histochemical staining using primary antibodies to BDCA2 (Mouse IgG1, Clone 124B3.13, Dilution 1:50, Dendritics, Lyon, France, EU).

The large majority of LE (68/75; 90.7%) and PLE biopsies (22/24; 91.7%) showed cutaneous infiltration of PDC, whereas no PDC were observed in normal skin. Cutaneous PDC infiltration was more frequent in CLE and PLE compared to SLE (respectively 96.4% 91.7% and vs 72.2%) and the PDC content was significantly higher in CLE and PLE compared to SLE ($p<0.05$).

This study confirms cutaneous PDC infiltration in the skin of LE and CLE patients and demonstrates their presence in the skin of PLE patients as never shown before, with a pattern of distribution similar to CLE patients.

Reflectance Confocal Microscopy for *in Vivo* Evaluation of Basal Cell Carcinoma's Surgical Margin

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Surgical excision represents the elective treatment for basal cell carcinoma (BCC). Several non invasive approaches have been proposed for *in vivo* determination of tumor margin, in the aim to develop a more rapid and less expensive approach comparable in results with Mohs micrographic surgery (MMS). Among them, reflectance confocal microscopy (RCM) seemed the most indicated for lateral margin detection in BCCs, due to its capability to explore the skin at cellular level resolution enabling the identification of tumor characteristic features

We proposed a new approach for lateral margin detection in BCCs through the combination of dermoscopy and RCM.

Ten patients with lesions clinically suggestive of non-pigmented BCCs with ill-defined margins were enrolled. All BCCs were first dermoscopically evaluated and the ill-defined margins were marked with a superficial cut and then inspected with RCM.

RCM evaluation showed BCC's foci beyond the pre-surgical marker in 3 out 10 lesions. Histology confirmed RCM results: presence of BCC features across the cut, corresponding to two superficial BCCs and a sclerodermiform-type BCC.

This new procedure helped to improve the identification of proper margins for surgical excision in non-pigmented BCC with clinically and dermoscopic ill-defined margins, reducing the number of stages in Mohs' surgery procedure.

Hydroa Vacciniforme: a Rare Photodermatosis EBV-Associated. Report of Two Clinical Cases

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Hydroa Vacciniforme (HV) is a rare photosensitivity disorder characterized by recurrent necrotic vesiculopapules on sun-exposed body sites, which heal with vacciniform scarring. HV usually starts during childhood and resolves spontaneously by early adult life and lesions are usually induced by sunlight and ultraviolet A irradiation. Diagnosis is based on using repetitive ultraviolet photoprovocative test to reproduce the characteristic lesions on a patient and on identifying the associated histological findings in a bioptic sample. Altough the precise etiology of HV is still unknown, several studies described the association between typical-HV and EBV latent infection and in these reports EBV was detected in patients' skin or mucosal lesions. We describe two cases of HV, that were diagnosed by reviewing the clinical features, laboratory and histopathological results and using UVA photoprovocative test.

New Nanovehicles for 5-ALA PDT Treatments: Formulation Development and Stability Studies

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5-ALA PTD has been approved by the FDA to treat pre-cancerous lesions such as actinic keratosis, and for the treatment of moderate to severe acne and in many other dermatological conditions. Moreover, 5-ALA PDT has been proposed also for skin aging treatments, to promote the renewal of epidermal cell by applying topical formulation containing lower concentration of 5-ALA (0.5% - 1%).

The aim of this study was to evaluate the stability and photostability of 5-aminolevulinic acid (5-ALA), both in solution and in finished topical products.

In particular we considered two innovative formulations with different applicative features: an oil in water liquid patented nanoemulsion, reliable for spray application, and a nanogel, a structured system the can be more easily used for the treatment of small areas. This kind of formulation should enhance the penetration of the active substance into the skin, and therefore make the treatment more effective. We firstly checked the UVA/UVB photosensitivity and the chemical stability of 5-ALA in aqueous solutions at various pH, temperature and incubation times, by means of UV-VIS and mass spectrometry spectroscopy, UCPL-DAD), in order to identify the main parameters to avoid rapid degradation of 5-ALA. Then we investigated the influence of 5-ALA concentration on the stability of the nanoemulsion, by means of Dynamic light scattering measures, at different time and storage temperatures. Finally we prepared nanogel systems by adding xanthan gum or hyaluronic acid to the nanoemulsion at different concentrations of 5-ALA (0.5-5%) and we evaluated their stability in time, by rheological tests.

The results show that 5-ALA is a highly reactive molecule: its stability in aqueous solution must be controlled by acidic pH (4.5) and low temperature of storage (4°C). The limited stability of 5-ALA in solutions implies low stability also in formulations, especially when high concentrations are used. Our results suggest that 5-ALA can be used up to 5% in the liquid nanoemulsion while the maximum concentration in the nanogel is 1%. Both the formulations guarantee the photostability of 5-ALA, that is high also in aqueous solutions.

PDT: MOLECULAR ASPECTS

Immunogenic Cell Death in Rose Bengal Acetate-PDT

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The new concept of immunogenic apoptosis or Immunogenic Cell Death (ICD) is recently integrating the tolerogenic apoptosis one. In this context, PhotoDynamic Therapy (PDT), an efficient cancer treatment, can give rise to abundant ICD and to immune response upon dead cells removal. ICD is associated with Damage Associated Molecular Patterns (DAMPs) exposure and/or release. The PhotoSensitizer (PS) up to now known to induce ICD are Photofrin, Hypericin, Foscan and 5-ALA. To increment the list of new PS ensuring ICD, we utilized Rose Bengal Acetate (RBAc) that is a powerful PS able to trigger apoptosis and autophagy by signals originating from or converging on almost all intracellular organelles. Here we investigated on the potential anti-cancer immunogenicity of RBAc-PDT on HeLa cells by screening the exposure and/or release of pivotal DAMPs, i.e. HSP70, HSP90, HMGB1 and calreticulin (CRT). We found that apoptotic HeLa cells after RBAc-PDT exposed and released high amount of HSP70, HSP90 and CRT early after the treatment. In particular, CRT displays an unevenly patched distribution on the cell surface. Conversely, autophagic HeLa cells after RBAc-PDT exposed and released HSP70, HSP90 but not CRT. Surface exposure and release of HSP70 and HSP90 are always higher on apoptotic than on autophagic cells. HMGB1 is released when secondary necrosis was present (24 h after RBAc-PDT). Very interestingly, RBAc-PDT is the first protocol of cancer PDT able to induce the translocation of HSP90 and co-expose ecto-CRT with ERp57. Altogether our data suggest that RBAc can be considered a suitable PS to ignite ICD during a PDT protocol.

Use of a Photosensitizer-NO Conjugate to Improve Photodynamic Therapy in Prostate Cancer Cells

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Prostate cancer (PCa) is the third leading cause of cancer-related deaths among men. Individuals who succumb to advanced stages of PCa inevitably become refractory to conventional therapy. Therefore, there is an urgent need to develop new drugs for these patients. While not currently used in the treatment of PCa, Photodynamic Therapy (PDT) has been successfully applied clinically in the management of both neoplastic and non-neoplastic diseases. We have reported the important role of Nitric Oxide (NO) in enhancing the PDT-mediated anti-tumor activity, both *in vitro* and *in vivo* as the result of the inhibition of the resistant NF-κB/YY1/RKIP loop in tumor cell lines. Hence, we hypothesized that chemical conjugates between photosensitizer (in this case pheophorbide *a*) and an NO donor may represent novel and potent cytotoxic agents against refractory PCa; in fact in this way it is possible to obtain simultaneously high production of reactive oxygen species and NO induction, increasing the oxidative damage due to PDT. Thus, we have evaluated *in vitro* the effectiveness of a new photosensitizer-NO derivative, namely DRPDT2, on cell growth and viability on human carcinoma prostate PC3 cell lines. Preliminary data show that DRPDT2 inhibits proliferation and induces cell death. Experiments on molecular signaling mediated by DRPDT2 are underway.

Cationic Antimicrobial Peptides are Efficient Carriers for the Delivery of Porphyrins to Cancer Cells

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Cationic antimicrobial peptides (CAMPs) are well known for their cytotoxic activity against various types of microorganisms. Since they exhibit several features typical of cell-penetrating peptides (CPPs) more recently are being considered also as carriers of anticancer drugs. Thus, we exploited the possibility of using CAMPs for the delivery of photosensitizers (PSs) with the aim to enhance the efficacy of photodynamic therapy (PDT) in killing cancer cells. Our model PS 5(4'-carboxyphenyl)-10,15,20-triphenylporphyrin (cTPP) was conjugated to the N-terminus of three different CAMPs, namely Buforin II, Magainin 2 and Apidaecin 1b and the delivery efficiency and phototoxicity of the conjugates (T-Buf, T-Mag, T-Api) were compared to that of the un-conjugated cTPP in A549 lung cancer cells *in vitro*.

Flow cytometry experiments showed that the kinetic of cellular uptake of the conjugates was very rapid and, after 5 h of incubation the uptake of T-Mag, T-Buf and T-Api was respectively 32, 8.5 and 6.5-fold higher than that of cTPP. Therefore, after cells irradiation with 1.5 J cm^{-2} of blue light, comparable photo-killing was measured with CAMP-porphyrin conjugates at nanomolar concentrations instead of micromolar concentrations as for the un-conjugated porphyrin. Confocal fluorescence microscopy showed T-Api inside the cell already after 1 h of incubation and Golgi apparatus localisation after 24 h. On the contrary T-Buf and T-Mag were associated to the plasma membrane after short incubations and showed punctuated cytoplasmic distribution without evident colocalization with particular compartments. Using inhibitors of specific endocytic pathways we found that the cell entrance of the conjugate depends on cholesterol and a lipid-raft mediated but caveolae-independent endocytosis is the predominant mechanisms. Gangliosides and sialic acid appear also to play a role in mediating the conjugate internalization. Notwithstanding the great improvement of PDT effects found in A549 cells using CAMP-porphyrin conjugates, further investigations are needed in order to determine the interactions of the drug conjugates also with non-cancerous cells in order to assess if our CAMP-conjugates displayed some selectivity toward malignancies as already reported for some un-conjugated CPPs and CAMPs.

5-ALA/PDT Induced Damage in Cells: Unravelling the Roles of p53 and ABCG2 Transporter

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Studying the effects of 5-ALA/PDT on two human lung cancer cell lines, namely A549 and H1299, we have made several observations at cellular and nuclear level. Reproducible and coherent effects on DNA were found only in H1299 cell line which p53 null. Indeed, the potentially detrimental effects of ROS on DNA produced by photosensitization of endogenously produced PpIX in cells have not been fully investigated up to now.

Beginning with the observation that 5-ALA/PDT induced S-phase accumulation in H1299 cells and not in A549, we explored the relative p21 protein levels and γ-H2AX phosphorylation status, performed comparative specific comet assays, evaluated the individual ATM/ATR and DNA-PK activation status. In whole the results sustained the exclusive occurrence of DNA damage and the activation of the relative signaling pathway only in H1299 cells. Fluorescence microscopy was used to investigate the PpIX localization. At variance with the A549 cells that restricted PpIX accumulation within the cytoplasm, the H1299 appeared to accumulate some photosensitizer within the nucleus. To explain such a difference we first have thought about the difference regarding p53 expression which is wild type in the A549 and null in the H1299 cells. Such possibility is concrete as the presence/absence of the p53 protein may have/have not preserved the DNA integrity in the cell lines. Such hypothesis, however has been questioned, on the basis of other observations made on two cell lines (respectively p53-wild type or –null). In these cases most of the DNA damage signs were not observed under ALA/PDT, independently from the p53 status.

Further experiments demonstrate that the expression of ABCG2, an efflux pump expressed in cell membranes, increased only in A549. Therefore the up-regulation of this transporter could inversely relate with the accumulation of intracellular PpIX and then with PDT efficiency. To finally demonstrate the ABCG2 involvement in the 5-ALA/PDT-induced DNA damage we are exploiting the properties of Ko143, a specific ABCG2 inhibitor. If this involvement will be positively demonstrated, the efficacy of 5-ALA/PDT could be tuned inhibiting the expression of the efflux pump.

ANTIMICROBIAL PDT

Photodynamic Treatment: How Do Prokaryotes Respond?

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Photodynamic Therapy (PDT) is a promising disinfection method potentially applicable to the treatment of localized bacterial infections easily accessible by light, as well as to the sanitization of inert devices. Therefore it is necessary to understand how prokaryotes respond to the photodynamic treatment in order to synthetize highly effective photosensitizers.

When bacteria are treated with a photosensitizer (PS), the irradiation with visible light favors the development of reactive species such as oxygen radicals, singlet oxygen and radicals that cause oxidative stress leading to cell death.

Besides the chemical structure of the PS and the microorganism features, it has been observed that the dark incubation, the PS concentration, the light energy density, the medium composition, the cell concentration and their physiological state, elicit different yield of photoinactivation, making a comparison difficult. Nevertheless the literature survey allows to individuate at least some relevant topics.

Among the bacterial features that influence PDT efficiency the cell wall structure seems to be a fundamental one, as Gram negative bacteria are usually less sensitive to PDT than the Gram positive ones. Satisfying results against Gram negative bacteria have however been obtained using PSs displaying a cationic net charge. Different killing yields have also been observed against strains belonging to the same species, suggesting that intraspecific differences in external components of the superficial bacterial structures can influence the interaction with the PS. An important role could also be played by the bacterial ability to activate a stress response to overcome the photooxidative stress.

Moreover most antimicrobial PDT experiments are performed against cell suspensions, a conditions that not always reflect the bacterial life style in natural contexts, where microorganisms often exist in complex communities, such as the biofilms. Therefore antimicrobial PDT should be also efficient in antibiofilm or antibiofouling treatment in clinical, industrial and environmental fields. The PSs should thus be able to diffuse across the complex biofilm matrix barrier rich in negatively charged organic compounds such as exopolysaccharides and DNA, in order to reach the deepest layers of biofilm and kill dormant cells, usually the most tolerant to antimicrobial treatments.

Synthesis of a series of BODIPYs, photodynamic activity and QSAR model

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Photodynamic therapy (PDT) is now a well-validated modality for the treatment of a number of cancer types. It is based on the systemic or topical administration of a non toxic compound, called photosensitizer (PS), followed by an incubation period to allow PS uptake by the tumor. The diseased area is then irradiated with low-energy, tissue penetrating light of the appropriate wavelength to activated the PS. Upon activation, the PS switches from its ground state to an excited singlet state, giving rise to a chain of electronic transitions that results in the production of cell death-inducing reactive oxygen species (ROS), mainly constituted by singlet oxygen ($^1O_2$).

Recently, one class of PSs has emerged, based on the boron dipyrromethene (BODIPY) fluorophore, exhibiting a number of features that might be exploited for PDT, as well as for photodiagnostic. both applied to oncology. BODIPY derivatives are easily synthesized in "one pot" procedure and their peculiar character is a high molar extinction coefficients and high quantum efficiencies of fluorescence (Φ_{fl}). It is known that high Φ_{fl} values are prejudicial to the photodynamic efficacy of any dyes which could be considered for PDT, therefore a rational modification of the scaffold, addressed to fluorescence inhibition, may afford an essential improvement of the photodynamic properties of the dyes.

In this work we synthesized a panel 25 BODIPYs with two iodine atoms in the 2, 6 positions of the pyrrole units and with various substituent in the 8 (*meso*) position. Each compound has been fully characterized by conventional analytical methods; furthermore the new compounds have been studied for their degree of lipophilicity and for the relative $^1O_2$ generation rate.

The in vitro photodynamic activity was evaluated on SKOV3 cell line through MTT test, characterized by 24h of dark incubation and 2h of irradiation with green LED. The results indicate that, on the whole, these molecules shown a promising activity in the range of nM concentrations, however the substituent in position 8 can influence the photodynamic activity outcome. For this reason the IC_{50} values obtained from the in vitro MTT assays have been used as input data for the development of a QSAR (Quantitative Structure Activity Relationships) model.

Proteins as biocompatible nanocarriers of hydrophobic photodynamic drugs: the complex of hypericin with β-lactoglobulin

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Using a combination of molecular modelling and spectroscopic experiments, the naturally-occurring pharmacologically active hypericin compound is shown to form a stable complex with the dimeric form of β-lactoglobulin (βLG). Binding is predicted to occur both at the narrow and, with lower affinity, at the wide clefts found at the interface between monomers in the dimeric βLG. The complex exhibits intense fluorescence emission and singlet oxygen photosensitising properties. The kinetic details of singlet oxygen production have been characterised and indicate that the protein scaffold protects hypericin from oxygen. The complex is active against *Staphylococcus aureus* bacteria and shows lower dark toxicity than free hypericin. Overall, the results are encouraging for pursuing the potential application of the complex between hypericin and βLG as a nanodevice with bactericidal properties.

PHOTOTHERMICS AND PHOTOACUSTICS

Single-Cell Manipulation in Femtosecond-Laser-Fabricated Optofluidic Devices

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Femtosecond-laser micromachining is an enabling technology that allows the realization of 3D devices for optofluidic applications. Direct femtosecond laser writing can be used both for the fabrication of high-quality waveguides, and, combined with chemical etching, for the realization of microfluidic channels. On-chip integration of optical waveguides intersecting the microfluidic channels makes it possible to implement photonic functionalities in lab-on-chips, as, for example, spatially-selective excitation of the fluorescence of the microchannel content. It also allows the realization of fully integrated optical traps and sorters for single cells. Thus femtosecond micromachining offers ideal capabilities for the realization of optofluidic devices for single-cell manipulation.

Nowadays, the main trend in optofluidics is towards full integration of the devices, so as to improve automation, compactness and portability. The current challenge in biology is the possibility to perform bioassays at the single cell level to unravel the hidden complexity in nominally homogeneous populations.

In this work, we first discuss the basics of femtosecond laser micromachining for the realization of optofluidic devices for biomedical applications. Then, we report on a new device implementing a fully integrated fluorescence-activated cell sorter. This non-invasive device is specifically designed to operate with a limited amount of cells but with a very high selectivity in the sorting process. Characterization of the device with beads and validation with human cells are presented.

The results obtained show the strong potential of this microfabrication technology, paving the way to increasingly compact and multifunctional optofluidic devices for the automated and reliable manipulation of biological samples with single cell accuracy.

Innovative Phototherapy for the Treatment of *Helicobacter pylori* Infection

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Helicobacter pylori (Hp) is a gram-negative microaerophilic bacterium that colonizes the mucus layer of the stomach with a prevalence of infection of more than the 50% in the world population. It is associated with chronic gastritis, peptic ulcer, MALT lymphoma, atrophic gastritis and gastric adenocarcinoma. Hp is considered a class 1 carcinogen agent by the World Health Organization.

Currently, Hp infection is treated with pharmacologic therapies, showing very high failure rates due to several side effects and antibiotic local resistance.

To overcome these limitations, the use of a photodynamic therapy has been explored. Till now endoscopic phototherapy trials have been performed by means of modified gastroscopes, exploiting the natural presence of porphyrins in Hp. However, this solution exhibits some clear disadvantages related to poor patient compliance and adverse effects.

Hereby we propose the design and characterization of an alternative solution for Hp phototherapy, based on (Light Emission Diode) LED technology. Two main aspects have been considered: (i) the study of light action spectrum for Hp phototherapy in the gastric mucosa environment. It has to be considered that light penetration depth in tissues varies from about 0.86mm at 405nm to 3.85mm for 630nm, being 405 and 630nm the two peak wavelengths of Hp porphyrin absorption. Interestingly enough, the most tissue penetrating light (630nm) corresponds to the secondary porphyrin absorption peak; (ii) the design and characterization of a device prototype, emitting the most appropriate wavelengths, with the desired power and intensity profile. Although it has been demonstrated that the most effective wavelength range for killing the bacterium *in vitro* is 375-425nm, we have also explored different wavelengths to which the photosensitizers are sensitive, in order to improve tissue penetration. Preliminary experiments have been performed with the LED module on cultured Hp, to verify the literature data and define the best irradiation parameters and geometry.

Optical Hyperthermia of Tumor Cells via Targeted Gold Nanorods

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Gold nanorods (GNRs) are an attractive nanomaterial for biomedical applications, displaying high light absorbance and scattering in the near-infrared region (NIR), provided an appropriate aspect ratio is conferred. The absorbed radiation may be efficiently converted into heat, so that GNRs are being considered for a broad variety of photothermal therapies such as the selective hyperthermia of cancer cells. In order to ensure specificity, GNRs can be functionalized with ligands to obtain specific binding to cancer cells, which lowers the thresholds of laser fluence required to destroy cancerous versus non-malignant cells.

In this study, GNRs were functionalized with a monoclonal antibody specific for Cancer Antigen 125 (CA 125), which is the marker actually used for diagnosis and management of ovarian cancer. *In vitro* application of GNRs for the targeting of cells overexpressing CA125 (HeLa) and for selective photothermal ablation of the same cells is reported. An optical and statistical method to assess targeting is presented.

The design of the probe was based on the pegylation of GNRs by polyethylene glycol (PEG) and further conjugation with antibodies against CA125, to provide targeting functionality. GNRs toxicity was evaluated by e.g. MTT assay. *In vitro* uptake of anti-CA125 GNRs was evaluated in HeLa and control HCT116 cells via dark field microscopy and silver staining studies. Selective targeting of HeLa cells after incubation of anti-CA125 GNRs with biological fluids such as serum and plasma was also verified in order to understand if the particles maintain their targeting capabilities even after systemic injection. The results confirmed that anti-CA125 GNRs were not cytotoxic and maintained targeting ability after biological fluid incubation. Efficacy of particle uptake was verified by darkfield imaging. Cell photothermal ablation by NIR laser irradiation was obtained with a lower energy than that required to kill control cells, due to the targeting efficiency.

PHOTOXICITY AND PHOTOSTABILITY

Transformation Processes of Drugs Occurring in Different Matrices

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Pharmaceuticals belong to a major class of emerging pollutants (EP) molecules in wastewater and surface water; in particular, antibiotic and anticancer drugs were identified as an important topic as, at present, the first ones are among major sources of bioactive molecules and, the second ones need to be overlooked owing to their cytotoxicity, genotoxicity, mutagenicity and teratogenicity. Even if their concentrations are low, the risk to humans is linked to a continuous and long term exposure via drinking water and food chain. The study of their degradation processes thus becomes important in understanding their toxic effects in the aquatic environment and, in particular, on human beings.

With this in mind, several widely diffused antibiotics, antiepileptic drugs, β-blockers and anticancer drugs were investigated. We focused on the photostability and photo-induced transformation pathways followed by drugs in water under UV-A or solar light, as photo-induced reactions are known to play a key role among the abiotic transformation following their discharge in the environment. Initially, laboratory experiments were performed to artificially produce degradation compounds similar to those formed in oxido/reductive pathways by adopting a photocatalytic process as a model-system and, afterwards, to identify them in real samples, beside parent compounds. Acute toxicity related to these EPs and to their transformation products was evaluated, too.

This approach has also been successfully used in the identification of new metabolic products, usable as biomarkers for the detection of illegal substances in biological matrices. Two cases are reported. Synephrine, an allowed substance, was considered to assess whether it may be a precursor to octopamine, a substance banned by WADA. With regard to sildenafil, a substance used to dope race-horses, new metabolic products were identified and characterized, whose presence has been detected in samples of urine and plasma of horses.

Photostability of Morphine and 6-MAM Exposed to Controlled UVB/UVA Irradiation in Water and Methanol Solution. Comparison with Data in the Solid State

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In the last years, many analytical methods based on liquid chromatography coupled with mass spectrometry detection have been developed to identify drugs of abuse and their metabolites. These studies are performed not only in clinical and forensic toxicology to investigate people drug use and abuse, but also in analyses of waters, especially in surface waters and sewage treatment plants, as they are new emerging environmental pollutants. Moreover, the quantification of these particular drugs and metabolites is of interest in the so called "sewage epidemiology", a novel approach for estimating drug consumption in a community by wastewater analysis.

The UVA and UVB light sensitivity of 6-monoacetylmorphine (6-MAM) and morphine, the main metabolites of heroin, has been studied in methanol, water solution and in the solid state by Liquid Chromatography - High Resolution, high accuracy Mass Spectrometry (LC-HRMS). The extent of photolysis in methanol/water solution and in the solid state was also calculated by LC-HRMS analysis.

The two analytes are quite stable under UVA light but very sensitive to UVB irradiation. In particular, they revealed to undergo a similar photodegradation pattern, both reaching 90% after 100 J/cm^2 of UVB irradiation in methanol. The photodegradation appears to be slightly faster for Morphine at lower doses. The photolysis rates in water are nearly one third lower than in methanol. In the solid state, the yield of photodegradation is lower than in solution. The description of possible structures of photoproducts obtained under UVB irradiation is presented. Photoaddition of the solvent and photooxidation seem to be the main pathways of phototransformation of these molecules and the same photoproducts due to oxidative process were identified in solution and the solid state.

A Study on Photodegradation of Methadone, EDDP and Other Drugs of Abuse in Hair Exposed to Controlled UVB Radiation and Comparison to Data Obtained in Methanol Solution

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In hair testing for drugs, knowledge on the effect of natural or artificial light on drug/metabolite contents has been useful to the forensic toxicologists, in particular when investigating drug concentrations above or below pre-determined cut-offs. The photodegradation (PD) of methadone and its metabolite 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) was studied by LC-High Resolution Mass Spectrometry (LC-HRMS) in authentic positive hair samples by comparison of drug concentrations before and after exposure to UVB light (*in vivo* study). The same approach was applied to opiates (6-monoacetylmorphine, 6-MAM, and morphine) and cocainics (cocaine and benzoylecgonine) in true positive hair. The PD yields were calculated for each drug class in 8 different positive hair samples irradiated by UVB at 300 J/cm^2, obtaining averages, ranges and standard deviations. Methadone was significantly affected by light (PD 55 % on average) while EDDP proved to be more photostable (PD 17 %). 6-MAM, morphine, benzoylecgonine, and cocaine resulted more photostable than methadone (PD on average, 21%, 17%, 20%, and 11% respectively).

In parallel, experiments on in vitro PD of the same analytes were performed irradiating pure methanol solutions of standards.

Differences were observed between the *in vivo* and the *in vitro* studies. Some factors possibly affecting the yields of PD in hair (and partially explaining differences *vs* methanol solutions studies) were also investigated such as the colour of hair (role of the melanins) and the integrity of the keratin matrix.

Photodamage in Two-Photon Optical Tomography of Human Skin

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A study on damage effects induced by femto-second laser radiation in thick samples of excised human skin tissue is presented. A two-photon laser scanning fluorescence microscope was used for damage creation and analysis. Besides healthy skin, different kinds of neoplastic lesions (benign nevi, basal-cell carcinomas and a melanoma) were studied comparatively. Identified damage mechanisms are based on photochemical, thermo-mechanical, and photoionization effects. They were identified via the induced changes in tissue structure and autofluorescence properties. Investigation of their dependences on laser power and exposure rate allowed to determine the underlying molecular processes.

Already at relatively low powers reversible and irreversible photobleaching effects were observed. Besides two-photon bleaching, also power dependences of 3rd and 4th order were found. Since direct absorption of 3 or more photons could be excluded, these processes indicate two photon, single photon subsequent absorption after a transition into the triplet state and double-biphotonic absorption, respectively.

At higher powers strong broadband luminescence set in, which indicates the formation of new compounds via photoionization. Finally, at highest powers strong disruptive cavitation occurred, which is of thermo-mechanical origin caused by single photon absorption in melanin. In comparative histological analyses, structural damages were found to be mostly limited to the biologically inactive surface layer stratum corneum, which is without medical implication.

All effects were evaluated also as a function of imaging depth and laser power in the different tissues. This allowed to obtain depth-dependent power limits for damage-free two-photon optical tomography. Guided by these values, the total penetration depth of this imaging technique could be improved via automatized in-depth laser power compensation.

Spectroscopic Investigation of the Molecular Interaction of New Potential Anticancer Drugs with DNA and Non-Ionic Micelles

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The interaction with DNA and with non-ionic micelles of new methyl-pyridinium and methyl-quinolinium derivatives, which showed good cytotoxicity in some cell lines, was investigated by UV-VIS spectroscopy and stationary fluorimetry. Also transient absorption spectroscopy with ns resolution, for detecting triplet and radicals formation, and with fs resolution, for ultrafast dynamics of singlet excited states, was performed. Spectrophotometric and fluorimetric titrations by adding salmon testes DNA provided evidence of a strong interaction between the quaternized azinium salts investigated and the polynucleotide, with binding constants of about $10^5 \div 10^6$ M^{-1}. Fluorimetric titrations showed a considerable enhancement of emission quantum yield, in agreement with a more rigid structure of the compounds involved in the binding with DNA. The predominant interaction mode, intercalation or groove binding, has been also investigated. Preliminary measurements by confocal fluorescence microscopy on cell lines in presence of the studied azinium salts will be performed with the aim to localize the compounds into cellular districts. Interaction between the azinium salts and some non-ionic surfactants was studied in order to find agents for drug solubilization and drug delivery. To check the ability of the micelles to encapsulate the studied salts into their hydrophobic core and to release them in presence of DNA, spectrophotometric and fluorimetric titrations were performed. Another proof of compounds encapsulation in micellar aggregates was obtained by ultrafast absorption spectroscopy, which demonstrated a slowdown of excited states dynamics in presence of surfactants, with respect to that of free compounds in aqueous medium. The obtained results suggested that the studied polar non-ionic micelles can act as promising drug delivery agents in order to minimize drug loss and degradation (preventing harmful side effects), to increase drug bioavailability and to release drugs into the cellular nuclei, allowing their interaction with DNA.

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Finito di stampare per conto della Fondazione Bruno Kessler
nel mese di giugno 2014
da Publistampa Arti Grafiche - Pergine Valsugana
su carta GardaPat 13 Kiara

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