UNIVERSITÀ DEGLI STUDI DI BARI

ISTITUTO PER I PROCESSI CHIMICO FISICI-CNR

CONGRESSO ANNUALE DELLA

SOCIETÀ ITALIANA DI FOTOBIOLOGIA

BARI, 11 – 13 GIUGNO 2015

SALA CONFERENZE

EX-PALAZZO DELLE POSTE

ATTI DEL CONGRESSO

Conference Program

Thursday 11th Environmental Photobiology

Chairperson: Gianfranco Canti

15.00 **Opening Session**

15:15 **Francesca Toma** (Berkeley, CA): *Towards Viable Artificial Photosynthetic Devices*
15:45 **Alessandra Antonucci** (Bari): *Bioconjugation strategy to covalently bind photosynthetic reaction centers (RCs) to hydrogen-bonded organic semiconductors*
16:00 **Simona la Gatta** (Bari): *Heptamethine cyanine dyes: a promising class of light harvesting antennas for biohybrid photosynthetic assembly*
16:15 **Andrea Meneghesso** (Padova): *Light use optimization of microalgae for biofuels production: Nannochloropsis gaditana acclimation to different light intensities*
16:30 **Marco Malferrari** (Bologna): *Bacterial photosynthetic reaction centers exposed to high temperatures for weeks retain their native structure and photoactivity when embedded into solid disaccharide matrices*

16:45 **Coffee Break**

Chairperson: Giorgia Miolo

17:15 **Davide Vione** (Torino): *Virus photoinactivation in surface-water environments*
17:45 **Eugenio Fazio** (Roma): *Biomorphic Optical Functions in Vegetable Seeds*
18:00 **Orietta Veronico** (Bari): *Tomato plant response under atmospheric particulate matter stress*
18:15 **Simone Bramuzzo** (Padova): *Control of the populations of Diptera vectors of pathogenic agents by environmentally friendly photodynamic processes*
18:30 **Francesca Italiano** (Bari): *Biosynthesis of Monodisperse Gold Nanoparticles by Rhodobacter sphaeroides*

Friday 12th Photomedicine

Chairperson: Giuseppe Palumbo

9:00 **Olimpia Coppellotti** (Padova): *Ricordo del professor Jori*
9:15 **Alessia Pacifico** (Roma): *Exposure to artificial ultraviolet sources: risks and benefits*
9:45 **Federica Barra** (Napoli): *Advances in understanding the roles of p53 and ABCG2 transporter in 5-ALA photodynamic therapy*
10:00 **Nora Bloise** (Pavia): *Low-Level Laser Irradiation: a tool for enhancing cell proliferation and osteogenic differentiation*
10:15 **Valentina Rapozzi** (Udine): *Molecular pathways in the response of tumors to photodynamic therapy: Role of NF-kB/YY1/RKIP loop*
10:30 **Monica Camerin** (Padova): *Bimodal cancer therapy using functionalized photoactivatable nanoparticles: in vitro and in vivo studies*

10:45 **Coffee Break**

Chairperson: Marina Venturini

11:15 **Nino Russo** (Rende): *The contribution of quantum chemistry to the design of new drugs active in photodynamic therapy*
11:45 **Antonino Mazzaglia** (Messina): *Supramolecular Assemblies based on Amphiphilic Cyclodextrins and BODIPY dyes as Potential Novel Photosensitisers for PDT*
12:00 **Giuliana Mion** (Trieste): *Phototoxic activity of new water soluble porphyrins and their Re(I) conjugates*

12:15 **Francesca Moret** (Padova): *Pluronic® P123/F127 mixed micelles as nanocarriers for benzoporphyrin derivatives in photodynamic therapy of cancer*
12:30 **Vito Rizzi** (Bari) *Rational Design of a Nucleoside-based Probe for Singlet State Oxygen Detection: Photostability and Mechanism of Reaction*

12:45 **Lunch Break**

13:45 **Poster Session**

Chairperson: Elena Reddi

14:30 **Evelyne Sage** (Parigi): *DNA damage induced by UVA radiation: a role in solar mutagenesis and carcinogenesis*
15:00 **Giorgia Miolo** (Padova): *Photobiological properties of 3-psoralenacetic acids*
15:15 **Marina Venturini** (Brescia): *Oral polipodium leucomotos increases the anti-inflammatory and melanogenetic responses of the skin to different modalities of sun exposures*
15:30 **Viviana Orlandi** (Varese): *Pigmentation affects the response to antimicrobial photodynamic therapy*
15:45 **Enrico Caruso** (Varese): *Cationic polymers enhance the antimicrobial photoinactivation induced by BODIPYs*

16:00 **Coffee Break**

Chairperson: Valentina Rapozzi

16:30 **Salvatore Sortino** (Catania): *Multifunctional Nanoparticles for Photoactivated Therapy*
17:00 **Giovanni Romano** (Firenze): *Action spectrum determination for the phototherapy of H. pylori infection in stomach tissue models*
17:15 **Paola Semeraro** (Bari): *In vitro studies of Chl a/CDs systems for PDT applications*
17:30 **Elena Reddi** (Padova): *Uptake and photo-toxicity of meta-tetra(hydroxyphenyl)-chlorin (m-THPC), Foslip® and Fospeg® in tumour cell sphaeroides*
17:45 **Gianpiero Valente** (Bari): *Silica Coated PbS Nanocrystals with Tunable Emission in the Near Infrared Region conjugated with RGD Peptide for Molecular Targeted Imaging*

Saturday 13th Materials and Techniques for Photobiology

Chairperson: Franco Fusi

9:00 **Francesco Vanzi** (Firenze): *Single molecule study of prokaryotic gene expression regulation*
9:30 **Gerardo Abbandonato** (Pisa): *Dual Probe for living cells sensing*
9:45 **Barbara Storti** (Pisa): *Unveiling the Spatio-Temporal Organization of TRPV1 Nociceptor in Live Cell Membranes*
10:00 **Emiliano Altamura** (Bari): *Giant vesicles as compartmentalized bio-reactors: optical spectroscopy investigations*
10:15 **Rosa Anna Pucciarelli** (Accademia di Belle Arti, Bari): *La luce da Caravaggio al contemporaneo*

10:45 **Coffee Break**

Chairperson: Giovanni Romano

11:15 **Roberto Improta** (Napoli): *The molecular mechanism of photodimerization in DNA*
11:45 **Cosima Damiana Calvano** (Bari)**:** *MALDI-ToF/ToF mass spectrometry analysis of intact bacteriochlorophylls by using 1,5-diaminonaphthalene as electron-transfer secondary reaction matrix*
12:00 **Elisa Panzarini** (Lecce): *Nanotechnology-based cancer photodynamic therapy*
12:15 **Vincenzo De Leo** (Bari): *Near Infrared emitting PbS-lipid nanocarrier for bioimaging applications*
12:30 **Closing remarks**

Environmental Photobiology

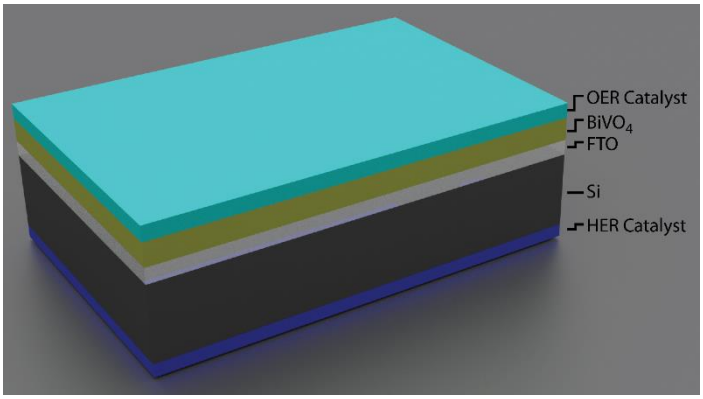
Plenary lectures and Communications

Towards Viable Artificial Photosynthetic Devices

Francesca M. Toma

Joint Center for Artificial Photosynthesis, LBNL, Berkeley, California 94720, United States
Chemical Science Division, LBNL, Berkeley, California 94720, United States

For the fabrication of an integrated monolithic device able to perform autonomous water splitting, different components should be interfaced together in an orchestrated manner. Photoelectrodes need to absorb in the visible range, with a valence and a conduction band suited for this reaction.1 Moreover, the multielectron – proton coupled processes involved in the semi-reduction and semi-oxidation reactions necessitate the presence of catalysts that manage the intrinsic hurdle of artificial photosynthesis.2,3 Herein, we address the study of the major challenges, namely performance, stability, and interfaces with catalyst and electrolytes, to enable implementation of lower band gap materials in water splitting devices.4, 5, 6, 7, 8



Schematic representation of the monolithically integrated device for artificial water splitting.

1. Y. Park et al, Chem. Soc. Rev. 42, 2321 (2012).
2. F.M. Toma et al, Nature Chem. 2, 826 (2010)
3. A. Sartorel et al, En. Env. Science 5, 5592 (2012)
4. J. Yang et al, J. Am. Chem. Soc. 136, 6191 (2014)
5. J. K. Cooper et al, Chem Mater 26, 5365 (2014)
6. L. Chen et al, ChemSusChem 8, 1066 (2015)
7. Y. Li, J. Phys. Chem. Lett 6, 493 (2015)
8. J. K. Cooper et al, J. Phys. Chem. C 119, 2969 (2015)

Virus photoinactivation in surface-water environments

Davide Vione[1], Michael Jon Mattle[2], and Tamar Kohn[2]

[1]University of Torino, Department of Chemistry, Via P. Giuria 5, 10125 Torino, Italy. Ph. +39-011-6705296. E-mail: davide.vione@unito.it
[2] Laboratory of Environmental Chemistry, School of Architecture, Civil and Environmental Engineering (ENAC), ÉcolePolytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

One of the processes that can inactivate viruses in surface waters is represented by photochemical reactions. A first photochemical inactivation pathway is the direct absorption of sunlight by the viruses, which occurs predominantly or almost exclusively in the UVB range. In such wavelength interval, sunlight absorption by viruses is often accounted for by the nucleic acid. Furthermore, indirect inactivation by sunlight is also possible [1].

Indirect phototransformation, which has been largely studied in the case of pollutants, involves reactive transient species such as $^{\bullet}OH$, $CO_3^{-\bullet}$, $^1O_2$ and the triplet states of chromophoric dissolved organic matter (^{3}CDOM*). The transients are generated by irradiation of photosensitisers such as CDOM (producing ^{3}CDOM*, $^1O_2$ and $^{\bullet}OH$), nitrate and nitrite ($^{\bullet}OH$) [2]. The radical $CO_3^{-\bullet}$ is produced *via* oxidation of carbonate/bicarbonate by $^{\bullet}OH$ and, to a lesser extent, upon oxidation of carbonate by ^{3}CDOM* [3]. The transient species formed by irradiated photosensitisers can induce the degradation of naturally occurring compounds, of pollutants, as well as photoinactivation of viruses [1,4].

The direct and indirect photochemical reactions in surface waters can be modelled, on the basis of photochemical reactivity parameters of the relevant molecules (direct photolysis quantum yield and second-order reaction rate constants with $^{\bullet}OH$, $CO_3^{-\bullet}$, $^1O_2$ and ^{3}CDOM*) and of water chemical composition [5]. The model predictions have been validated against field data of pollutant phototransformation. They can also be applied in the case of viruses [1]. A software tool has recently been derived from the model (APEX: Aqueous Photochemistry of Environmentally-occurring Xenobiotics), and it is available for free download [6].

Here we show photoinactivation results for the model viruses MS2 (RNA phage) and PhiX174 (DNA phage). A major issue for modelling was the laboratory measurement of the photoinactivation quantum yield of both viruses (as opposed to the widely employed but much less useful action spectrum) and of the second-order reaction rate constants with $^{\bullet}OH$, $CO_3^{-\bullet}$, $^1O_2$ and ^{3}CDOM*. To measure the second-order rate constants, the virus photoinactivation kinetics (loss of its ability to infect *E. coli*) was compared with the degradation kinetics of model molecules (tryptophan for $^{\bullet}OH$, tyrosine for $CO_3^{-\bullet}$, furfuryl alcohol for $^1O_2$, while the use of anthraquinone-2-sulfonate as CDOM proxy does not require the use of model molecules [7]). The combination of measured photoinactivation parameters and of photochemical modelling with APEX suggests that MS2 would be degraded both directly, and indirectly upon reaction with $^1O_2$ (the latter increasing in importance with increasing water depth and dissolved organic carbon). In contrast, PhiX174 would mainly undergo direct photoinactivation [8]. The modelling results are in very good agreement with field data concerning coliphage photoinactivation in an artificial wetland [1].

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2. S. Halladja, A. Ter Halle, J.P. Aguer, A. Boulkamh, C. Richard, *Environ. Sci. Technol.* **2007**, *41*, 6066.
3. S. Canonica, T. Kohn, M. Mac, F.J. Real, J. Wirz, U. von Gunten, *Environ. Sci. Technol.* **2005**, *39*, 9182.
4. E. De Laurentiis, S. Chiron, S. Kouras-Hadef, C. Richard, M. Minella, V. Maurino, C. Minero, D. Vione, *Environ. Sci. Technol.* **2012**, *46*, 8164.
5. D. Vione, M. Minella, V. Maurino, C. Minero, *Chemistry Eur. J.* **2014**, *20*, 10590.
6. M. Bodrato, D. Vione, *Environ. Sci.: Processes Impacts* **2014**, *16*, 732.
7. A. Bedini, E. De Laurentiis, D. Vione et al., *Photochem. Photobiol. Sci.* **2012**, *11*, 1445.
8. M. J. Mattle, D. Vione, T. Kohn, *Environ. Sci. Technol.* **2015**, *49*, 334.

Bioconjugation strategy to covalently bind photosynthetic reaction centers (RCs) to hydrogen-bonded organic semiconductors

Alessandra Antonucci[1], Rocco Roberto Tangorra[1], Halime Coskun[2], Dominik Farka[2], Yasin Kanbur[2], Alessandra Operamolla[1], Francesco Milano[3], Eric Daniel Glowacki[2], Serdar Sariciftci[2], Massimo Trotta[3], Gianluca M.Farinola[1]

[1] Università degli Studi di Bari Aldo Moro, Dipartimento di Chimica, Via Orabona 4, 70125 - Bari, Italy, 0805442027
[2] Linz Institute for Organic Solar Cells (LIOS), Physical Chemistry, Johannes Kepler University, Linz (Austria)
[3] CNR-IPCF UOS Bari, Via Orabona 4, 70125 - Bari, Italy, 0805442027

Photosynthesis represents one of the most important biological reactions in the biosphere, since all life on Earth, directly or indirectly, depends on it as a source of energy. Nature performs the photosynthetic process using specialized protein-pigments complexes organized to ensure up to 98% conversion of the absorbed photons in stable, long-living charge separated states. A proper combination of the photosynthetic core, the reaction center (RC), with engineered materials, *i.e.* metals or inorganic semiconductor electrodes, has attracted great attention for the building of new versatile hybrid devices for solar energy conversion[1]. Here we propose a covalent approach able to stably anchor RCs onto evaporated thin films of the hydrogen-bonded pigments epindolidione (EPI) and quinacridone (QNC)[2], a well-known class of organic colorants which have recently emerged as promising semiconductors, demonstrating hole mobility in the range of 0.1 – 1 cm^2/Vs and outstanding operational stability in both air and aqueous media with pH 3-10. Due to low-toxicity, biocompatibility and potential low-cost, EPI and QNC substrates are envisioned to go where other traditional semiconductors simply cannot be applied, resulting to be amenable to direct surface bioconjugation. The NH functional group of these molecules in thin film reacts spontaneously with N-hydroxysuccinimide functionalized linkers as disuccinimidyl suberate. The protruding linkers are then used to covalently bind the lysines residues of the *Rhodobacter sphaeroides* reaction center, by forming an amide linkage.

Our protocol is shown to preserve the semiconducting properties of the pigments while maintaining the protein's photoactivity. Multiple-reflection infrared spectroscopy and atomic force microscopy demonstrated the effective covalent binding and the robustness of the protein anchoring even after buffer washing procedures compared to the weakness of the physisorbed RC interactions. Furthermore, RC charge recombination kinetic measurements confirmed the fully functionality of bioconjugated proteins and ruled out any possible hindering effect from the organic films. As key results of our work, we have shown that semiconductors preserve their favorable electrical properties: the proposed photoconductor devices operate under water, before and after RCs anchoring. These are enabling steps for using hydrogen-bonded pigments as a platform for multifunctional bioelectronics devices, paving the way in designing and realizing new photosynthetic protein-based hybrid systems.

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2. E. D. Głowacki et al., *Adv. Mater.,* **25**, 1563 – 1569 (2013).

Heptamethine cyanine dyes: a promising class of light harvesting antennas for biohybrid photosynthetic assembly

R. Ragni,[1] S. La Gatta,[1] L. Lepore,[1] F. Milano,[2] O. Hassan,[3] A. Operamolla,[1] A. Agostiano,[1] G.M. Farinola[1] and M. Trotta[2]

[1] *University of Bari "Aldo Moro", Department of Chemistry, Via Orabona 4, 70126 Bari, Italy*
[2] *Istituto per i Processi Chimico Fisici-CNR, Department of Chemistry, Via Orabona 4, 70126 Bari, Italy*
[3] *Istituto di Chimica dei Composti Organometallici-CNR, Department of Chemistry, Via Orabona 4, 70126 Bari, Italy*

The bioconjugation of photosynthetic proteins with efficient organic light harvesting antennas is a very intriguing approach to build novel hybrid organic-biological machineries that, mimicking nature, employ solar energy to generate photocurrents or to drive thermodynamically unfavoured reactions, reaching efficiencies higher than those obtainable by their natural conterparts. Such hybrid systems are potentially useful as active materials in new generation devices for photovoltaics and biosensing.
In the frame of our studies on organic-biological hybrids for solar energy conversion,[1] here we present the design, synthesis and preliminary characterization of a series of heptamethine cyanine dyes (such as Cian-1 in Figure 1a) particularly suitable as light harvesting antennas for the photosynthetic Reaction Center (RC) of the purple bacterium *Rhodobacter sphaeroides* strain R26. These molecules have been properly tailored to have efficient light absorption in the visible spectral range, where the RC absorbance is very low, and efficient emission in the near infrared region, in correspondence of the highest RC absorption peaks (Figure 1b).[2] Moreover, the charged sites within their molecular structure make these molecules highly soluble in detergent aqueous environment where the RC is stable, this allowing them to approach the bioconjugation sites of the protein. Finally, the synthesized cyanines are endowed with a carboxylic moiety useful for their covalent binding to the amino groups of the RC lysine residues. Our preliminary results show that the bioconjugation of these organic antennas to the RC is expected to be a very profitable strategy to afford highly efficient organic-biological hybrids for solar energy conversion.

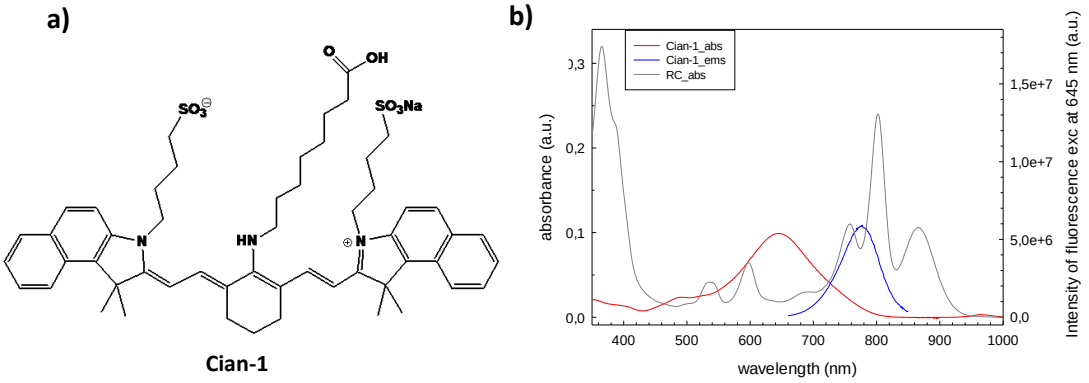

Figure 1

1. F. Milano, R. R. Tangorra, O. Hassan Omar, R. Ragni, A. Operamolla, A. Agostiano, G. M. Farinola, M. Trotta, *Angew. Chem. Int. Ed.*, **2012**, 51, 11019-11023.
2. X. Peng, F. Song, E. Lu, Y. Wang, W. Zhou, J. Fan, Y. Gao, *J. AM. Chem. Soc.*, **2005**, 127, 4170-4171.

Light use optimization of microalgae for biofuels production*:* *Nannochloropsis gaditana* acclimation to different light intensities

Andrea Meneghesso[1], Nicoletta La Rocca[1], Giovanni Finazzi[2] and Tomas Morosinotto[1]

[1]*University of Padova, Department of Biology, Via Ugo Bassi 58b 3512, Italy, +390498276325*
[2] *Institut de Recherches en Technologies et Sciences pour le Vivant - CEA Grenoble, France*

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 biofuel outdoor productions where illumination conditions are highly variable.
Nannochloropsis gaditana belongs to Eustigmatophyceae, a class of eukaryotic algae resulting from a secondary endosymbiotic event. Species of this class have been poorly characterized thus far but are now raising increasing interest in the scientific community because of their possible application in biofuel production.
In this work we analyzed different responses of *Nannochloropsis gaditana* to the exposition to light of different intensities. Electron microscopy shows clear differences in the thylakoids ultrastructure during photoacclimation to low or high light. The remodeling of photosynthetic apparatus was investigated using various spectroscopic approaches showing that acclimatative response in *Nannochloropsis* results in a variation of photosystems stoichiometry as well as modulation of antenna size in both photosystems. Changes in photosynthetic apparatus composition are accompanied by an increased ability to transfer electrons, also thanks to the contribution of cyclic electron transport.

Bacterial photosynthetic reaction centers exposed to high temperatures for weeks retain their native structure and photoactivity when embedded into solid disaccharide matrices

Marco Malferrari[1], Francesco Francia[1] and Giovanni Venturoli[1,2]

[1] Laboratorio di Biochimica e Biofisica Molecolare, Dipartimento di Farmacia e Biotecnologie, FaBiT, Università di Bologna.
[2] Consorzio Nazionale Interuniversitario per le Scienze Fisiche della Materia (CNISM), c/o Dipartimento di Fisica, Università di Bologna.

Protein internal dynamics is known to be inhibited in glassy matrices upon dehydration, both in the absence or in the presence of glass forming disaccharides [1,2], like trehalose, resulting in increased protein thermal stability. To get insight into such matrix effects we have compared the retardation of large-scale protein dynamics associated with protein denaturation in photosynthetic bacterial reaction centers (RC) dehydrated at controlled relative humidity in the absence (RC films) or in the presence of trehalose (RC-trehalose glasses). Isothermal denaturation kinetics have been obtained at 44°C by analyzing the temperature induced alterations of the Q_x and Q_y bands of the RC bacteriochlorin cofactors, as a function of the sugar/protein molar ratio, *m*, varied between 0 and 10^4. In dehydrated RC films we found that exposure to high temperature causes the release of bacteriochlorin cofactors and carotenoids from their binding pockets, on the timescale of a few hours, as compared to tens of minutes in liquid suspensions [3]. The release of cofactors implies temperature-induced alterations at the level of the tertiary structure. Upon increasing *m*, loss of the native structure is slowed progressively, and above *m* ~500 the RC is stable for weeks. We propose that the ability of trehalose matrices to protect against thermal denaturation stems mainly from its propensity to form stable, extended networks of multiple hydrogen bonds, connecting residual water at the protein-matrix interface simultaneously with surface residues of the RC complex and trehalose molecules of the matrix. Trehalose is also expected to reduce the free volume fraction of protein alone matrices (RC films), thus reducing the motional degrees of freedom of the whole system.

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2. Cordone, L., et al. (2005), *Biochim Biophys Acta Proteins Proteomics* 1749, 252-281.
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Biomorphic Optical Functions in Vegetable Seeds

E. Fazio[1], L. Silvestroni[2] and S. Palleschi[3]

[1]Sapienza Università di Roma, Dept. Fundamental and Applied Sciences for Engineering, Via A. Scarpa 16, 00161 Roma, Italy, Tel: +39.331.62.41.019, eugenio.fazio@uniroma1.it
[2]Sapienza Università di Roma, Dept. of Experimental Medicine, Viale Regina Elena 324, 00161 Roma, Italy, Tel:+39.06.44.57.885, leopoldo.silvestroni@uniroma1.it
[3]Istituto Superiore di Sanità, Department of Haematology, Oncology and Molecular Medicine, Viale Regina Elena 299, 00161 Roma, Italy, simonetta.palleschi@iss.it

Biophoton emission has been largely investigated during the last century. It was reported with different names, ranging from bio-photons to dark-luminescence, to ultraweak light emission and so on… All such names describe the capability of biological structures to emit light in specific environments or when the biological structures are somehow stressed. *The discovery of biophoton emission was performed by Alexander Gurvich [1], who introduced two fundamental ideas on its origin: A) biophotons emanate from free-radical reactions and B) the emission in the life science comes from an organised "morphogenetic" field at the basis of life. Such theory further bifurcated into two different approaches: a mechanistic one (biophotons as a sign of random metabolic imperfections) and a vitalistic one (biophotons generated by quantum coherent states) [2].*
Here we report the biophoton emission from germinating seeds (more specifically cannellini beans). Analysis of biophotons emitted during imbibition by "vital" as well as "denaturated" parts of seeds reveals both coherent and incoherent statistics. Moreover each part of the bean has specific light emission characteristics which differ from the other parts and from the whole seed.
Is there any specific reason for such light emission? Is it just a chemical "waist", a funny phenomenon or it is functional for the vital life of the seed and the future plant? Internal emitted light remains inside the seed: thus, seeds should act like resonators or optical concentrators for biophotons. We have analysed cannellini beans from geometrical points of view, in order to characterise their shape and their optical behaviours. The very peculiar geometry of seeds allows the light to travel within each bean like within an integrated waveguide. Cannellini bean shape is indeed not symmetrical, with a characteristic deformation towards the hemisphere containing the embryo and its plumula. Such asymmetry is indeed not casual: instead it has specific functionalities to collect energy and send it towards specific areas inside the seed, in order to control the whole germination process. Light is used by the seed as a tool to control all activities, to inhibit or to start germination [3].

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3. E. Fazio, L. Silvestroni and S. Palleschi, *Biomorphic Optical Functions in Vegetable Seeds*, submitted

Tomato plant response under atmospheric particulate matter stress

Barbara Elisabetta Daresta[1]*, Francesca Italiano[2]*, Gianluigi de Gennaro[1,3], Massimo Trotta[2], Maria Tutino[1] and Pasqua Veronico[4]

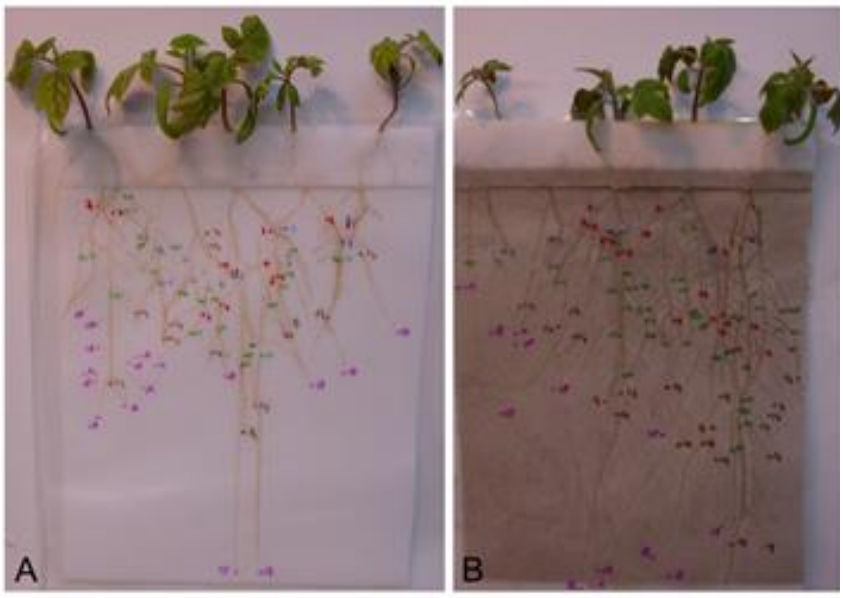
[1]ARPA Puglia, Corso Trieste 27, 70126 Bari, Italy, +39 080 5460362

[2]CNR, Istituto per i Processi Chimico Fisici, via Orabona 4, 70125 Bari, Italy, +39 080 5442033

[3]Dipartimento di Chimica Università degli Studi di Bari, via Orabona 4, 70125 Bari, Italy, +39 080 5543254

[4]CNR, Istituto per la Protezione delle Piante, via G. Amendola 122/d, 70126 Bari, Italy, +39 080 5583377

Urban particulate matter (PM) can affect green plants either via deposition on the above-ground biomass, where the contaminants can penetrate the leaf surface, or indirectly via soil–root interaction. In our investigation, a model experiment was carried out to demonstrate the direct effect of PM on tomato (*Solanum lycopersicum* L.) plant growth. A monitoring campaign of PM10 was conducted at an urban background site of Canosa (Apulia, Southern Italy) in four different days (1, 2, 3, 4). PM10 samples were collected for 24 hours on quartz fiber filter. The filters were then cut into two parts, one of which was used for the chemical characterization of the PM10 and one for the growth of tomato. Organic and elemental carbon and polycyclic aromatic hydrocarbons (PAHs) content were analysed for all the tested filters. Tomato plants were grown for 18 days directly on filters absorbed with PM10. The germination rate of tomato seeds and some parameters of seedlings primary growth of this plant species (length of root and shoot, their fresh weight and content of photosynthetic pigments in shoot) were used as laboratory indicators of phytotoxicity.



Growth pouches holding 5 tomato (*Solanum lycopersicum* L.) seedlings, photographed 18 days after seedling transfer from

Substantial differences were found in the growth of root apparatus respect to that of control plants. A significant decrease of primary root elongation, a large amount of secondary roots and a decrease in plant and root weights were found. To assess if the direct exposition of roots to PM10 induced an oxidative stress, reactive oxygen species (ROS) concentration was evaluated by measuring the fluorescence arising from oxidation of DCFH-DA in both control and treated roots. Quantitative analysis of ROS indicated that an oxidative burst in response to abiotic stress occurred in roots directly grown on PM10, whose detrimental effect was also confirmed by the findings on chlorophyll content and chlorophyll-to-carotenoid ratio.

1. Daresta et al. Atmospheric particulate matter (PM) effect on the growth of *Solanum lycopersicum* cv. Roma plants. Chemosphere 119 (2015) 37–42

Control of the populations of Diptera vectors of pathogenic agents by environmentally friendly photodynamic processes

Laura Guidolin, Simone Bramuzzo, Monica Camerin, Olimpia Coppellotti

Department of Biology, University of Padova, Via U. Bassi 58/B, 35121 Padova, Italy

The photodynamic inactivation of microbial pathogens represents a very flexible technology which has a broad scope and potential for tackling a range of problems connected with the microbiological contamination of waters of different origin and physical or chemical characteristics. In this way it is possible to achieve a thorough control of the population of a variety of harmful bacteria, fungi and protozoa by using a single protocol. The need to control pathogens involved in the transmission of serious diseases, e.g. malaria, Dengue, Nile fever and Chikungunya, is becoming, in part because of global warming, overpopulation and globalization, an increasingly urgent challenge even in those countries considered so far safe from these diseases.

The exploitation of photodynamic processes, through the use of natural and environmentally safe photosensitizers, could represent an attractive solution for controlling the population of larvae of Diptera (e.g. *Aedes* and *Anopheles*) which are carriers of *Plasmodium* or other agents, responsible for these pathologies.

Previous studies in our laboratory indicate that a porphyrin and a chlorin can be good candidates to carry out this task. Fluorescence spectrophotometer assay and fluorescence microscope analysis indicate that these compounds are well ingested from larvae when proposed in combination with protein-rich food; larvae exhibit a high mortality at low concentrations after few hours of irradiation with either artificial light (400-800 nm) or sunlight, while gradual photobleaching prevents their accumulation in the environment and transmission in the food chain.

Toxicity tests on non-target organisms such as *Tetrahymena thermophila* for chlorin and *Tetrahymena thermophila* and *Daphnia magna* for porphyrin were negative; this indicates an excellent specificity in killing action of larvae, without involvement of non-target species.

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Biosynthesis of Monodisperse Gold Nanoparticles by *Rhodobacter sphaeroides*

Francesca Italiano[1], Angela Agostiano[1-3], Benny Danilo Belviso[2], Rocco Caliandro[2], Benedetta Carrozzini[2], Roberto Comparelli[3], Maria Teresa Melillo[4], Ernesto Mesto[5], Gioacchino Tempesta[5], Massimo Trotta[3]

[1] Università degli Studi di Bari "Aldo Moro", Dipartimento di Chimica, via E. Orabona, 4 – 70126 Bari, Italy
[2] CNR - Istituto di Cristallografia, Sezione di Bari, via G. Amendola, 122/O– 70126 Bari, Italy
[3] CNR - Istituto per i Processi Chimico-Fisici, Sezione di Bari, via E. Orabona, 4 – 70126 Bari, Italy
[4]CNR.-.Istituto per la Protezione Sostenibile delle Piante, Sezione di Bari, Via G. Amendola, 122/D 70126 Bari, Italy
[5] Università degli Studi di Bari "Aldo Moro", Dipartimento di Scienza della Terra e Geoambientali , Via E. Orabona 4, 70126 Bari, Italy.

Gold nanoparticles exhibit unique electronic, optical, and catalytic properties that are different from those of bulk metal and have several applications in optoelectronics, imaging technology, catalysis, and drug delivery. Currently, there is a growing need to develop eco-friendly nanoparticle synthesis processes using living organisms, such as bacteria, fungi and algae. In particular, microorganisms are well known to protect themselves from metal ion stress either by intracellular-segregation mechanism or by secreting them into the external medium. This defensive behaviour can be exploited to obtain a more efficient fabrication of advanced functional nanomaterials than chemical synthesis routes: biological syntheses do not require hazardous organic solvents and surfactants , and can work at environmental temperature and pressure, preserving high selectivity and reproducibility.
Rhodobacter sphaeroides is a facultative phototrophic anoxygenic proteobacterium known for its capacity to grow under a wide range of environmental conditions, with promising applications in bioremediation [1, 2].
The response of the photosynthetic bacterium *Rhodobacter sphaeroides* to gold exposure and its reducing capability of Au(III) to produce stable Au(0) nanoparticles is reported in this study. The properties of prepared nanoparticles were characterized by UV-Visible (UV-Vis) spectroscopy, Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy, Transmission Electron Microscopy (TEM), X-ray Photoelectron Spectroscopy (XPS), X-ray Fluorescence Spectrometry (XRF) and X-ray Absorption Spectroscopy (XAS) measurements. Gold nanoparticles (AuNPs) were spherical in shape with an average size of 10±3 nm. Based on our experiments, the particles were likely fabricated by the aid of reducing sugars present in the bacterial cell membrane and were capped by a protein/peptide coat. The nanoparticles were hydrophilic and resisted to aggregation for several months. Gold nanoparticles were also positively tested for their catalytic activity in nitroaromatic compounds degradation.

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Photomedicine

Plenary lectures and Communications

Exposure to artificial ultraviolet sources: risks and benefits

Alessia Pacifico

Phototherapy Unit, San Gallicano Dermatological Institute, IRCCS, Rome, Italy

Ultraviolet (UV) exposure is not only due to the sun but also to indoor tanning devices that have been shown to lead to an elevated risk of melanoma and non melanoma skin cancer (NMSC). Indoor tanning is a widespread practice and in the last few years, more and more people, especially teenagers and women, are exposed to high radiant exposures of UV thorough artificial sources. UV radiation causes DNA damage, inflammation, erythema, sunburn, immunosuppression, photoaging, gene mutations and skin cancer. Several studies indicate that genetic alterations in the p53 tumor suppressor gene play an important role in the development of skin cancer. The p53 protein is also involved in programmed cell death and it has been proposed that p53 serves as a "guardian of genome" by aiding DNA repair or causing elimination of cells with excessive DNA damage. Chronic UV exposure, overwhelms DNA repair mechanisms leading to induction of p53 mutations. Keratinocytes carrying p53 mutations acquire a growth advantage by virtue of their increased resistance to apoptosis and resistance to cell death is a key event in photocarcinogenesis. Apoptosis-resistant keratinocytes undergo clonal expansion that may lead to formation of actinic keratoses and squamous cell carcinomas. Because UV-induced p53 mutations arise early during the development of skin cancer, discontinuation of UV treatment can still result in skin tumor development, although the kinetics of tumor occurrence is delayed in the latter case. In conclusion, cancer development can be delayed but not abrogated upon further avoidance of exposure to UV radiation.

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The contribution of quantum chemistry to the design of new drugs active in photodynamic therapy

Marta Alberto[1], Bruna Desimone,[1], Gloria Mazzone[1], and Nino Russo[1]

[1] Università della Calabria, Dipartimento di Chimica e Tecnologie Chimiche, Via P. Bucci, cubo 14c, 87036 Rende, Italy, Phone: +390984492106

The possibility to design new photosensitizers active in photodynamic therapy starting from computed electronic and geometrical properties by using density functional theory should be presented. In particular, we will show as the main photophysical properties that a drug active in photodynamic therapy must possess (absorption wavelengths shifted in the Near Infrared Region, singlet-triplet energy gaps and spin-orbit matrix elements large enough to allow an efficient intersystem spin crossing) can be reliably predicted by modern density functional methods. The studied systems include isoindole BODIPY, squaraine, porphycene, bare and metallated porphyrin-like systems able to activate singlet O_2 excited state (Type II reactions).

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DNA damage induced by UVA radiation: a role in solar mutagenesis and carcinogenesis

Evelyne Sage

Institut Curie, CNRS UMR3347, Centre Universitaire, 91405 Orsay, France, 33-(0)1 69 86 71 87

The solar UV radiation reaching Earth's surface comprises above 90% of UVA radiation (320-400 nm). UVA contributes to photodermatosis, skin aging, and likely to skin carcinogenesis. It has long been recognized that UVA radiation induces the formation of reactive oxygen species (ROS), mainly singlet oxygen, generating an oxidative stress in cells. UVA produces a variety of damage to DNA, proteins, lipids, that elicites complex cellular responses. Cyclobutane pyrimidine dimers (CPDs) and 8oxoguanine are the two major DNA lesions produced by UVA radiation. We and others observed that, CPDs prevail in mammalian cells, whereas 8oxoG predominates in yeast. The predominant occurrence of CPDs at TT sites could suggests that CPDs are formed by photosensitization via triplet state energy transfer. However, recent evidences are in favor of CPDs formation by direct absorption of UVA photons by DNA. UVA exposure, *via* reactive oxygen species, causes an extended S-phase and a slowing-down of DNA replication, affecting various parameters of DNA replication while inducing extensive protein oxidation and glutathionylation. These events may have implication in the fate of skin cells. UVA is a relatively weak mutagenic agent, in agreement with a low induction of DNA damage [1]. UVA fingerprint in genome is in fact the typical UV mutagenic signature, C to T transition at bipyrimidine sites, described in mammalian cells or skin. Melanoma cells have been recently reported to harbor such mutations with a high frequency, and UVA could well contribute to these mutations. In addition, the use of sunbed (> 99% UVA) has been associated with a significant increase in the risk of melanoma. Collectively, these observations suggest that the role of UVA in photocarcinogenesis may have been underestimated. They also point out a need for better understanding the UVA genotoxicity and for a better photoprotection against UVA radiation.

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Multifunctional Nanoparticles for Photoactivated Therapy

Salvatore Sortino

Laboratory of Photochemistry, Department of Drug Sciences, University of Catania, Viale Andrea Doria 6, 95125 Catania.
Email: ssortino@unict.it

The achievement of nanoparticles (NPs) able to release therapeutic species in a photo-controlled fashion is a major challenge in the burgeoning field of nanomedicine. Light is in fact a powerful tool for the introduction of bio-active agents in a cellular environment, mimicking an "*optical microsyringe*" with an exquisite control of three main factors, site, timing and dosage, which are determining for the therapeutic outcome.[1] Moreover light-triggering is biofriendly and offers the additional advantages of not affecting important physiological parameters such as temperature, pH and ionic strength. Singlet oxygen ($^1O_2$) and nitric oxide radical (NO) are two main cytotoxic species, which can be photogenerated by means of suitable photosensitizers. Common to $^1O_2$ and NO is the capability to attack biological substrates of different nature (*i.e.,* lipids, proteins, and DNA), representing multitarget therapeutic agents and avoiding Multiple Drug Resistance problems encountered with several conventional drugs often target-specific. Moreover, due to their short half-life and lack of charge, both $^1O_2$ and NO radical diffuse in the cellular environment over short distances without inflicting systemic side effects common to general anticancer drugs. For all these reasons, the combination of $^1O_2$ with NO has received growing attention in the last few years[2] with the exciting prospect to tackle cancer diseases. In our laboratories, we have been working on the design and fabrication of a number of engineered, fluorescent nanoparticles able to photogenerate $^1O_2$, NO or both under either one- and two-photon excitation. This contribution illustrates some of the most recent and representative examples including polymer nanoparticles, polymer micelles and carbon dots, highlighting the rationale design and their potential relevance in biomedical research.

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Advances in understanding the roles of p53 and ABCG2 transporter in 5-ALA photodynamic therapy

Ilaria Postiglione[1], Federica Barra[1], and Giuseppe Palumbo[1]

[1]University of Naples "Federico II", Department of Molecular Medicine and Medical Biotechnologies, via S. Pansini, 5 - 80131, Naples, Italy, 081/7462947

Studying the effects of 5-ALA/PDT on two human lung cancer cell lines, namely A549 and H1299, we have made and reported several observations at cellular and nuclear level including reproducible and coherent effects on DNA but only in H1299 cell line. At glance, we considered that this different behavior was related to p53 status/expression which, at variance with the H1299 cells where it is "null", is fully functional in the A549. To confirm such hypothesis, two identical lines of human colorectal adenocarcinoma, the HCT-116, which differ only for the expression of p53 due to a knocking-down of the gene itself (HCT +/+ and HCT -/-), were subjected to the same treatments as above and fully analyzed. In both cases, although to different extents, PDT treatment caused DNA damage, suggesting that the DNA injury is not exclusively dependent on presence or absence of an active form of p53 but, presumably, also by other effectors.

Searching for these effectors, we observed that the expression of ABCG2, an efflux pump expressed in cell membranes, is increased during photo-activation but only in A549 cells. Therefore, we hypothesized that up-regulation of this transporter inversely controls the accumulation of intracellular PpIX and then, the PDT efficiency. The ABCG2 involvement in the 5-ALA/PDT-induced DNA damage was finally demonstrated taking advantage from the properties of a specific ABCG2 inhibitor (Ko143). By inhibiting this transporter even A549 cells, subjected to 5-ALA/PDT presented clear signs of DNA damage as directly confirmed by Comet Assay analysis.

Currently we are analyzing other panels of cells differing for the expression of both genes, namely p53 and ABCG2 (++,+-,--,-+) to evaluate which combination is more effective/ineffective in inducing DNA damage. This information may provide valuable advice about the level of efficacy of the treatment on specific cancer cells, then tangible clue on effectiveness of therapy.

Low-Level Laser Irradiation: a tool for enhancing cell proliferation and osteogenic differentiation

Bloise N[1,2], Ceccarelli G[2,3], Minzoni P[4], Mantelli M[5], Cusella De Angelis M[2,3] and Visai L.[1,2,6]

[1]University of Pavia, Department of Molecular Medicine,Via Taramelli, 3/B- 27100, Pavia, Italy
2Center for Tissue Engineering (CIT), Via Ferrata 1, 27100, Italy
[3]University of Pavia, Department of Public Health, Experimental Medicine, Via Forlanini,8-27100 Pavia, Italy
[4]University of Pavia, Department of Electrical, Computer, and Biomedical Engineering, Via Ferrata, 5 A I-27100 Pavia, Italy
[5]Pediatric Oncohematology Laboratory, Policlinico San Matteo Foundation, IRCCS, Viale Golgi, 19-27100, Pavia, Italy;
[6]University of Pavia, Department of Occupational Medicine, Ergonomy and Disability, Via Forlanini, 2710 and Salvatore Maugeri Foundation, IRCCS, Via S. Boezio 28, 27100 Pavia, Italy

Over the last year, it has been highlighted that physical factors, like biochemical factors, can modulate and reprogram cell function. In this context, it has reported that low-level laser therapy (LLLT) promotes bone regeneration and influences the behaviour of many cell types, including mesenchymal stem cells (MSCs) although the exact mechanisms involved remain poorly understood[1,2].The objective of this study was the investigation of low-level laser therapy potentiality on proliferation and differentiation of human osteoblast-like cells (SAOS-2) in the absence/presence of osteogenic factors. The effects of laser on proliferation were assessed daily up to seven days of culture in cells irradiated once or for three consecutive days with different laser doses (1-3 J/cm^2). The obtained results showed that laser stimulation enhances the proliferation potential of Saos-2 cells without changing their telomerase pattern or morphological characteristics. The effects on cell differentiation were assessed after three consecutive laser irradiation treatments in the presence or absence of osteoinductive factors on day 14. Enhanced secretion of proteins specific for differentiation toward bone as well as calcium deposition and alkaline phosphatase activity were observed in irradiated cells cultured in a medium not supplemented with osteogenic factors. All these data represent an argument supporting the power of low-level laser treatment on bone regeneration. Therefore, the following step was the application of laser on human bone marrow mesenchymal stem stem (hBM-MSCs) and then the evaluation of its effects on hBM-MSCs behaviours prior the implantation to bone defect. Our preliminary results showed that the laser application influences the proliferation potential of hMSCs by modulation cell-cycle gene expression and production of IGF-1, which is reported to regulate stem cell proliferation and differentiation. Currently, we are performing a comparative study in order to understand further the effects of laser irradiation at different energy doses on the human mesenchymal stem cells differentiation towards osteogenic phenotype and as well as the biochemical mechanism underlying these effects. Taken together these findings suggest a possibility of using LLLI as a “photoceutical” for *in vitro* stem cells preconditioning prior to transplantation in order to enhance their helpful application on bone tissue regeneration

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Molecular pathways in the response of tumors to photodynamic therapy: Role of NF-kB/YY1/RKIP loop

Emilia Della Pietra[1], Daniela Cesselli[1], Benjamin Bonavida[2], Luigi Emilio Xodo[1] and Valentina Rapozzi[1]

[1]University of Udine, Departmen of medical and Biological Sciences, P.le Kolbe 4, 33100, Udine, Phone +39 0432 494394
[2]Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, Jonsson Comprehensive Cancer Center, University of California Los Angeles

Photodynamic therapy (PDT) is a clinically approved, minimally invasive therapeutic treatment that exerts a selectively cytotoxic activity towards cancer cells. This technique involves administration of a photosensitizer followed by irradiation at a wavelength corresponding to its absorbance band. In the presence of oxygen, a cascade of stress oxidative reactions lead to direct tumor cell death, damage to the microvasculature and induction of a local inflammatory reaction. In addition to the production of singlet oxygen and reactive oxygen species, PDT can induce the release of nitric oxide (NO) by up-regulating nitric oxide synthases (NOS). Since non-optimal PDT often causes tumor recurrence, understanding of the molecular pathways involved in the photoprocess is a challenging task for scientists. The present study has examined the response of the PC3 human metastatic prostate cancer cell line, following repeated low-dose pheophorbide *a* treatments, mimicking non-optimal PDT treatment. The analysis was focused on the NF-kB/YY1/RKIP circuitry as it is (i) dysregulated in cancer cells (ii) modulated by NO and (iii) correlated with the epithelial to mesenchymal transition (EMT). We hypothesized that a repeated treatment of non-optimal PDT induces low levels of NO that lead to cell growth and EMT via regulation of the above circuitry. The expressions of gene products involved in the circuitry and in EMT were analyzed by western blot. The findings demonstrate the cytoprotective role of NO following non-optimal PDT treatments that was corroborated by the use of L-NAME, an inhibitor of NOS.

Bimodal cancer therapy using functionalized photoactivatable nanoparticles: *in vitro* and *in vivo* studies

Monica Camerin[1], Aurore Fraix[2], Paola Irato[1], Giovanna Sotgiu[3], Greta Varchi[3], Ilse Manet[3], Salvatore Sortino[2], Olimpia Coppellotti[1]

[1] Department of Biology, University of Padova, Via U. Bassi 58/B, 35121 Padova, Italy
[2] Laboratory of Photochemistry, Department of Drug Sciences, University of Catania, Viale A. Doria 6, 95125 Catania, Italy
[3] Italian National Research Council, Institute for the Organic Synthesis and Photoreactivity, Via Gobetti 101, 40129 Bologna, Italy

Photodynamic therapy (PDT) is a well-established modality for the cancer treatment through the synergy of three essential nontoxic components: photosensitizer (PS), light of the appropriate wavelength to activate the PS and molecular oxygen which is the terminal generator of toxic species, like singlet oxygen ($^1O_2$). Combined modality therapies represent a promising strategy for the improvement of photodynamic therapy efficacy. Nitric oxide (NO) has been proven to have a role in the bioregulation of several functions, but also plays a very promising anticancer activity via mechanisms involving different subcellular targets as compared with $^1O_2$. Thus, NO can act in synergy with $^1O_2$ for a new bimodal anticancer phototherapy. Different nanoparticles (NPs) have been decorated with PS and NO donor species enabling the photorelease of $^1O_2$ and NO. Here we report the results obtained with two polymeric core-shell nanoparticles electrostatically decorated with meso-tetra(4-sulfonatophenyl)porphyrin (TPPS) as PS and electrostatically or covalently decorated with the 4-nitro-3-(trifluoromethyl)phenyl-amino group as NO donor.

In our laboratory we have tested two different photoactivable nanoconstructs with bimodal therapeutic effects based on the photo-controlled generation of reactive oxygen species (ROS) and reactive nitrogen oxide species (RNOS). *In vitro* studies on B78H1 cell line (an amelanotic clone of murine melanoma) showed that the treatment with the two different nanoparticles induced a significant higher cytotoxicity but the synergistic effects are more important for nanoparticles having both the TPPS and the NO-donor electrostatically bound to the NPs cationic shell. The suitability of the polymeric NPs to act as an appropriate delivery system for cancer cell treatment was also demonstrated by fluorescence microscopy.

To test the possible extension of combined PDT to *in vivo* systems, female C57BL/6 mice bearing a subcutaneously transplanted amelanotic melanoma were used as experimental models. Pharmacokinetic studies were performed at different times to evaluate the uptake as a function of injection time.

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Supramolecular Assemblies based on Amphiphilic Cyclodextrins and BODIPY dyes as Potential Novel Photosensitisers for PDT

Giuseppe Sortino[1], Enrico Caruso[2], Miryam Malacarne[2], Stefano Banfi[2] and Antonino Mazzaglia[1]

[1]CNR-ISMN c/o Dip. Scienze Chimiche dell'Università di Messina, Viale Ferdinando Stagno D'Alcontres 31, 98166 Messina, Italy

[2]Dipartimento di Scienze Teoriche ed Applicate (DiSTA), - Università dell'Insubria di Varese, Via Dunant 3, 21100 Varese, Italy

Amphiphilic cyclodextrins (ACyDs) are an intriguing class of carrier systems which, recently, have been proposed to deliver porphyrinoids both for potential application in PDT [1] and for photo-anticancer indipendent effect [2]. Generally, the design of supramolecular complexes between nanoaggregates based on cationic or non ionic ACyD and useful photosensitisers (PSs) aims to preserve the PDT efficacy of PS, reducing the tendency of PS to self-aggregate, without affecting the quantum yield of singlet oxygen ($^1O_2$). Actually, at suitable PS/carrier molar ratio (considering a carrier excess), ACyD could assist the disaggregation of the PSs self-assembly, increasing the contents of monomer which has a higher photoactivity with respect to supramolecular self-oligomers. In this communication, we focus on supramolecular assemblies between a novel class of BODIPY dyes (BL01 and BL01I) and non-ionic ACyD (heptakis(2-O-oligo(ethyleneoxide)-6-hexylthio)-β-CD, SC6OH). Nanoassemblies of SC6OH/BODIPY at 1:2 molar ratio ([BODIPY] = 200 μM) have been prepared by hydratation with aqueous solution (PBS, pH 7.4) of an organic film (obtained by slow evaporation of ACyD and BODIPY solutions in DCM) and following sonication. The supramolecular assemblies have been studied with complementary tecniques such as UV-Vis, steady-state fluorescence spectroscopy, anisotropy, resonance light scattering (RLS) and characterized, in order to elucidate size, drug loading and sites of PS entrapment in ACyD nanoaggregates. SC6OH nanoaggregates (≅ 100 nm) form stable complexes with both BL01 and BL01I, decreasing the amount of photobleached products upon green light irradiation (Green LED 12 W, light dose 33 J/cm^2). In detail, the presence of BL01 self-oligomers in DCM is mantained both in PBS and in aqueous solution, as detected by RLS.

The photodynamic activity have been studied on adenocarcinoma cell line (HCT116) with a standardized protocol (24 h of incubation time with photosensitizer, 2 h of irradiation with green LED). In these conditions BL01 shows a low activity but this result can be explained by a low quantum singlet oxygen generation, that is characteristic of this kind of guest molecules. Instead of BL01I presents two iodine atom in the BODIPY core and this presence causes the heavy atom effect that increase the singlet oxygen generation and, consequently, photodynamic activity of the compound. Also, the photoactivity and the cellular uptake of the complexes were investigated and, very interestingly, these properties are comparable with free BL01 and BL01I. Altogether, our evidences agree with the feasibility of these systems as promising novel tools in PDT.

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Phototoxic activity of new water soluble porphyrins and their Re(I) conjugates

Giuliana Mion,[1] Teresa Gianferrara,[1] Alberta Bergamo,[2] Gilles Gasser,[3] Vanessa Pierroz,[4] Enzo Alessio[1]

[1]*Department of Pharmaceutical and Chemical Sciences, University of Trieste, P.le Europa 1, 34127 Trieste, Italy, 0405582525.*

[2]*Callerio Foundation Onlus, Via A. Fleming 22-31, 34127 Trieste, Italy, 040 5588639, 040 569933*

[3]*Institute of Inorganic Chemistry, University of Zürich, Winterthurerstrasse 190, CH-8057, Zurich, Switzerland, +41 44 635 46 30.*

[4]*Institute of Molecular Cancer Research, University of Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland, +41 44 635 34 77*

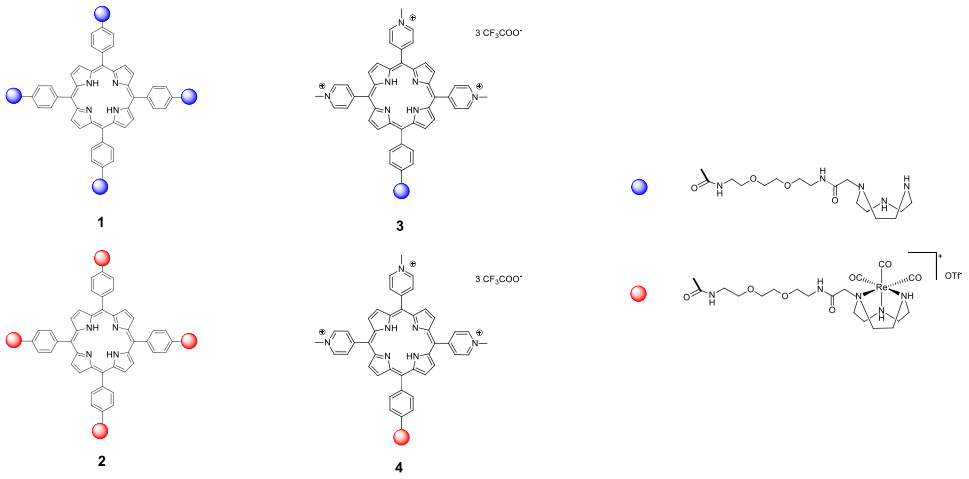
Water soluble synthetic porphyrins and metal-porphyrins have attracted considerable interest for their applications as photosensitizers, as they can be used for photodynamic therapy (PDT) and fluorescence imaging [1], and they can behave as carrier ligands for the transport of metal compounds into cancer cells.

Here we report two novel symmetric and asymmetric porphyrins **1** and **3**, and their corresponding Re(I) conjugates **2** and **4**.

The in vitro phototoxicity activity of these compounds was studied on two cell lines, HeLa human cervix cancer cells and H460M2 non-small cell lung cancer. All the compounds except **2** were not cytotoxic in the dark up to 100 µM. Compounds **1** and **2** revealed good phototoxic index (PI >77 on H460M2 cells (**1**) and PI >71 on HeLa (**2**) at 10 J/cm^2 and 650 nm), instead compound **3** and **4** have greater efficacy against the H460M2 tumor cell line with an IC_{50} of micromolar range.

The metal fragment seems to affect the phototoxicity activity of the symmetrical porphyrin **1**, while it seems not affect IC_{50} of the asymmetric porphyrin **3**.

Among the investigated porphyrins, the best singlet oxygen generator is **1,** while compounds **2**, **3** and **4** have lower singlet oxygen quantum yields. The intracellular localization of all the compounds was studied in HeLa cells by confocal fluorescence microscopy, and showed that all the porphyrins except **1** accumulate in the nucleus of HeLa cells.



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Pluronic® P123/F127 mixed micelles as nanocarriers for benzoporphyrin derivatives in photodynamic therapy of cancer

Francesca Moret[1], Diogo Silva Pellosi[2,3], André Luiz Tessaro[4], Elisa Gaio[1], Elena Reddi[1], Wilker Caetano[2], Fabiana Quaglia[3] and Noboru Hioka[2]

[1] University of Padova, Department of Biology, Via Ugo Bassi 58/B, 35121, Padova, Italy, 0498276335
[2] State University of Maringá, Chemistry Department, Av. Colombo 5.790, 87020-900, Maringá, Brazil
[3] University of Napoli Federico II, Department of Pharmacy, Via Domenico Montesano 49, 80131 Napoli, Italy
[4] Federal Technological University of Paraná, Chemistry Department, rua Marcílio Dias, 635. CEP 86812-460, Apucarana, Brazil.

The photosensitizer Benzoporphyrin derivative monoacid ring A (BPDMA, trade name Verteporfin®) in an aqueous liposomal formulation (trade name Visudyne®) is a FDA/US photosensitizer approved for the treatment of age-related macular degeneration. The synthetic route of BPD produces equimolar quantities of two benzoporphyrin regioisomers named as A-ring and B-ring (BPDMA and BPDMB) type derivatives. Despite possessing physicochemical properties and clinical activity similar to BPDMA, the B-ring derivative is not commercialized due to its high tendency to self-aggregate even in liposomes, impairing photodynamic efficiency. Thus, to improve the delivery of the benzoporphyrin derivatives and avoid aggregation, we developed several aqueous micellar formulations made of Pluronic® copolymers. These surfactants molecules contain two hydrophilic poly(ethylene oxide) (PEO) and one hydrophobic poly(propylene oxide) (PPO) regions arranged in a PEO-PPO-PEO triblock structure, and above its critical micellar concentration self-assemble in nanosized core–shell micelles, able to encapsulate drugs in the PPO micelle core and to expose PEO regions to impart stealthy properties to nanoparticles. BPDMA and BPDMB and BPDMA/BPDMB mixture (BPD-mixt) were formulated in Pluronic® P123 or F127 as well as P123/F127 mixtures. Only P123/F127 mixed micelles in the ratio 2:1 allowed the encapsulation of BPD as monomers improving loading, photophysical properties and stability during time, even under diluted conditions. Intracellular uptake and photo-toxicity of the P123/F127 mixed BPD formulations were evaluated i*n vitro* in HeLa and A549 cancer cells in comparison to the PS delivered without micelles. The results showed that, despite of the reduced uptake of the PS delivered by nanoparticles, the photo-activity of micellar BPDMA at low concentrations was higher than that of the free PS, suggesting the possibility to reduce the dose of administered drug while preserving efficacy. Furthermore, also the photo-activity of BPD-Mixt was comparable to that of BPDMA, while only BPDMB micelles were inefficient cell photosensitizers because of their poor uptake. Our findings suggested that the expensive procedure of BPD regioisomer separation can be bypassed by encapsulating both drugs in Pluronic® P123/F127 mixed micelles. Future perspectives are directed to broaden benzoporphyrin derivative application to cancer therapy by further improving the nanovehicle in order to obtain selective targeting of tumor cells as well as the possibility of co-delivery of photosensitizers and other drugs for combing PDT and chemotherapy.

Rational Design of a Nucleoside-based Probe for Singlet State Oxygen Detection: Photostability and Mechanism of Reaction

Vito Rizzi[1], Ilario Losito[1,3], Andrea Ventrella[1], Paola Fini[2], Paola Semeraro[1], Angela Agostiano[1,2], Francesco Longobardi[1], Pinalysa Cosma[1,2]

[1]Università degli Studi "Aldo Moro" di Bari, Dip. Chimica, Via Orabona, 4- 70126 Bari, Itayl, +390805443443
[2]Consiglio Nazionale delle Ricerche CNR-IPCF, UOS Bari, Via Orabona, 4- 70126 Bari, Italy, +390805442226
[3]Centro Interdipartimentale SMART, Via Orabona, 4- 70126 Bari, Italy, +390805442019

Light has played a therapeutic role for humans for centuries. Indeed, ancient Egyptian, Indian and Chinese populations used the exposure to sun to treat a variety of diseases, including vitiligo, psoriasis, cancer and even psychosis.[1] Among modern versions of such a therapeutic approach, the Photo Dynamic Therapy (PDT), *i.e.* the combination of a light source with a photosensitizing agent and endogenous molecular oxygen, has emerged as a therapy for cancer and for hyperproliferative, ophthalmic and dermatologic diseases in the last 30 years and is currently feasible in several medical institutions around the world.[2] When excited by a low-energy and tissue-penetrating radiation having an appropriate wavelength, the photosensitizer (PS), localized in a specific tissue/cell, is able to produce Reactive Oxygen Species (ROS) from molecular oxygen through photochemical reactions. The study of the generation and reactivity of ROS during a PDT process is thus extremely important.[3] The most studied ROS, considered the major responsible of the peroxidation of proteins within a cell, is Singlet State Oxygen ($^1O_2$).[4] Although further reactive species can be present during a PDT treatment, they can be difficult to detect, due to the *in vivo* capture by a variety of antioxidants, making their lifetime very short. It is thus essential to develop appropriate methodologies for their detection. Fluorescent probes are excellent sensors of ROS due to their high sensitivity, simplicity in data d on the interaction between S^4TdR and singlet oxygen, generated by Rose Bengal (RB), chosen as a modcollection and high spatial resolution in microscopic imaging techniques, but their major drawback is the risk of interference. Consequently, an investigation on a novel probe, potentially able to detect ROS, *i.e.* 4-Thiothymidine (S^4TdR), has been recently undertaken in our laboratories. In spite of the remarkable amount of research involving S^4TdR, only limited information is available on its behavior in an aqueous environment (representing a model system for more complex biological environments). Consequently, a comprehensive study has been carried out on this aspect in the first part of the investigation, using several complementary techniques, namely UV-VIS, FTIR and 1H-NMR spectroscopies and ElectroSpray Ionization Mass Spectrometry (ESI-MS)[5]. In the second step, using the same techniques, the study has been focuseel ROS. The main results obtained for this process will be thus the object of the present communication.

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Photobiological properties of 3-psoralenacetic acids

Giorgia Miolo, Adriana Chilin, Giovanni Marzaro, Lisa Dalla Via

Department of Pharmaceutical and Pharmacological Sciences, University of Padova, via Marzolo 5, I-35121 Padova

Some 4,8-dimethyl-3-psoralenacetic acids with different substitutions in the furan ring were synthesized and studied. All the planned psoralenacetic acids bear methyl groups in the 4 and 8 positions of the benzopyrone nucleus and are variousy substituted at the furan ring: compound **1** and **2** bear one or two methyl groups the 4' and in the 5' position, respectively; compound **3** presents a bulky tert-butyl in the 4' position, while compound **4** presents the two positions of the furan ring substituted with a further condensed cyclohexane nucleus. These psoralenacetic acids showed to be a novel class of psoralen derivatives characterized by an interesting photobiological profile. The carboxylic group at the 3 position, useful to confer hydrophilic properties, appeared to be detrimental for the classical intercalation into DNA, because of repulsive interactions with the positive surface of the macromolecule. Nevertheless, the new derivatives possess a notable photoantiproliferative activity, due to a peculiar mechanism of action consisting in a decarboxylation step before exerting their photobiological activity. The most active compound 2 is able to induce a noteworthy photocytotoxic effect, with GI50 values submicromolar on human tumor cell lines, and none effect in the dark. The involvement of DNA photodamage and ROS formation after UVA light-mediated decarboxylation are responsible for its biological activity, as demonstrated comparing the activity profile of the decarboxylated analogue.

In conclusion, compound 2 could thus be considered as a prodrug, inactive without UVA light but activated upon specific irradiation, thus preventing unselective side effects, opening new perspective for agents to be employed in PUVA therapy.

Oral polipodium leucomotos increases the anti-inflammatory and melanogenetic responses of the skin to different modalities of sun exposures

Venturini Marina, Zanca Arianna, Rossi Mariateresa, Arisi Mariachiara and Calzavara-Pinton Piergiacomo

University of Brescia, Department of Dermatology, Spedali Civili di Brescia, p.le Spedali Civili, 1 – 25123 Brescia, Italy

Background: Oral supplementation of several botanical antioxidants demonstrated to increase the minimal erythemal dose (MED) but the effects on the time course of the inflammatory reaction and on the intensity and time course of the tanning response are still unknown.
Objective: To assess spectrophotometrically the intensity and time course of the inflammatory and tanning responses to increasing dosages of solar simulated radiation (SSR) at baseline and after oral supplementation of an extract of Polipodium Leucotomos (PLE).
Materials and Methods: This is a single-centre, non-randomized intra-patient clinical trial enrolling 10 healthy subjects. They underwent phototesting with SSR and intensity and time course of erythemal and pigmentary reactions were assessed visually and spettrophotometrically after delivery of minimally, sub- and super- erythemal doses for 7 consecutive days at baseline and after 15 days of daily oral supplementation of 480 mg of PLE.
Results: PLE supplementation was followed by a significant increase of the minimal erythema dose (MED), without significant changes of the minimal melanogenic dose (MMD). Spettrophotometric assessment of the Δa^* parameter in test areas exposed to equally erythemogenic or sub-erythemogenic doses did not show differences whereas a significantly faster recovery of the inflammatory reaction following the delivery of super-erythemal doses was seen after PLE supplementation. The melanin Index (MI*) was higher under all conditions of exposure.
Conclusions: oral PLE supplementation increased the MED and, if equally super-erythemogenic doses were delivered, the recovery of the inflammatory reaction was faster. In contrast, the melanogenic threshold was not changed whereas, if equally erythemogenic doses were delivered, the tanning response was stronger after PLE supplementation.

Pigmentation affects the response to antimicrobial photodynamic therapy

Orlandi V.T., Bolognese F., Barbieri P.

University of Insubria, Department of Theoretical and Applied Sciences, via J.H. Dunant 3, 21100 Varese, Italy

P. aeruginosa is a well-known opportunistic pathogen that may worsen infected patient conditions due to the ability to produce many virulence factors and to resist to many antibiotics and disinfectants. Among antimicrobial approaches emerging in the last years, the photodynamic therapy (PDT) seems to be a promising technique to be combined to the traditional chemotherapy. PDT exploits the photo-oxidative stress elicited by photosensitizers (PSs) exogenously administered to bacteria; PSs absorb visible light and cause the arise of ROS (Reactive Oxygen Species) by energy transfer or electron flow causing cell death.

P. aeruginosa can produce a variety of pigments that contribute in different way to some features and behaviors of the bacterium. In this study, we investigated the correlation between the type and amount of typical *P. aeruginosa* pigments and the tolerance to photo-inactivation. We observed that experimental conditions influenced the pigment production and yield that, in turn, affected the PDT efficiency, not only in *P. aeruginosa* PAO1 wild type strain, but also in the isogenic derivatives with altered pigmentation.

The pigmentation combined with the ability to elicit an oxidative stress response may contribute to the survival to the photo-oxidative stress.

Cationic polymers enhance the antimicrobial photoinactivation induced by BODIPYs

Enrico Caruso[1], Viviana Orlandi[1], Stefano Banfi[1], Paola Barbieri[1], Barbara Leva[1], Stefano Ferrara[1], Amedea Manfredi[2]

[1]University of Insubria, Department of Theoretical and Applied Sciences, Via H.J. Dunat 3, 21100 Varese, Italy, mail: enrico.caruso@uninsubria.it

[2]University of Milan, Department of Chemistry, Via Golgi 19, 21133 Milano, Italy

Traditional antimicrobial approaches are often ineffective in environmental and clinical field. In recent years novel, convenient and inexpensive methods for removing microbial contamination have been developed. Antimicrobial photodynamic therapy (APDT) seems to be very promising for the efficient inactivation of pathogenic microorganisms localized on an inert or biological surface. In APDT, the photosensitizer and a low energy light source are provided together to induce oxidative stress in bacteria.

Recently, besides the more commonly used porphyrins and phthalocynines, an alternative class of photosensitizers (PSs) has emerged, based on the boron dipyrromethene (BODIPY) fluorophore. This class of PSs features a number of chemico-physical properties that have been exploited for PDT, as well as for photodiagnosis both applied to oncology. Furthermore BODIPY derivatives are easily synthesized in “one pot” procedure and their peculiar character is a high molar extinction coefficient and high quantum efficiency of fluorescence (Φ_{fl}). It is known that high Φ_{fl} values are essential for diagnosis but are prejudicial to the photodynamic efficacy, 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 study we analyzed the influence of some polycationic polymers on the photodynamic stress elicited by a BODIPY. The polymers belong to the family of the poly-amidoamines (PAA) which are biocompatible macromolecules characterized by an increasing number of cationic charges depending on the molecular weight.

Two microorganisms have been chosen as representative of Gram-negative and Gram-positive bacteria, *Escherichia coli* and *Staphylococcus aureus*. The photoinactivation of these strains has been performed by irradiation of the cultures in the presence of BODIPY alone or in the presence of both BODIPY and PAA. The PS concentration was very low and PAA was not toxic to cells. We observed with both microorganisms an enhancement of the killing yield at least of two log units. Future investigations will be addressed to understand the role of PAA in making more efficient the photoinactivation by BODIPY.

Action spectrum determination for the phototherapy of *H. pylori* infection in stomach tissue models

Giovanni Romano[1], Silvia Calusi[1], Barbara Orsini[1], Giuseppe Tortora[2], Alessandro Filomena[3], Alessio Gnerucci[1] and Franco Fusi[1]

[1]University of Florence, Department of Clinical and Experimental Biomedical Sciences "Mario Serio", viale Pieraccini 6, 50139 Florence, Italy, 0552758431
[2] The BioRobotics Institute, Scuola Superiore Sant'Anna, Pisa, Italy
[3]ASL 10, Firenze, Italy

In the framework of the growing rate of antibiotic resistance, new therapeutic solutions are being considered against bacterial infections, among which photodynamic therapy (PDT) is certainly a very attractive perspective[1].
In recent years, innovative solutions for endoscopic illumination have been defined and studied in many applications, e.g. in the case of catheter infections, lung and pancreas tumours, stomach infections by *H. pylori*. One of the most important studies necessary to optimize PDT efficacy is the analysis of the light action spectrum, which in turn depends on multiple factors among which (i) the photosensitizer absorption spectrum ; (ii) the tissue absorption and scattering properties.
In the literature many studies have been performed on this subject, concentrating mainly on one of those aspects[2,3]. In this communication, we will show recent results obtained for the determination of the light action spectrum for bacterial eradication by PDT, in the case of *H. pylori*. Our approach is based first on the knowledge of the stomach mucosa optical properties: data coming from optical microscopy (*ex vivo* mucosa samples) and literature results have been combined to perform Monte Carlo simulations of the light transmitted and reflected by simple models of stomach *plicae*. In this way it was possible to reproduce the *in vivo* illumination conditions due to a light source of given spectrum. Secondly we have considered the type(s) and absorption spectrum of the endogenous photosensitizers present in *H.pylori*. By merging the two datasets we have obtained an estimate of the light action spectrum for the phototherapy of *H.pylori* by means of a semi-theoretical approach.

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In vitro studies of Chl *a*/CDs systems for PDT applications

P. Semeraro[1], P. Fini[2], G. A. Chimienti[3], M. F. Gargano[1], E. Altamura[1], V. Rizzi[1], A. Agostiano[1], P. Cosma[1]

[1]*University of Bari, Department of Chemistry, Via Orabona, 4, 70125, Italy, +39 0805443443*
[2]*CNR-IPCF, UOS Bari, Department of Chemistry, Via Orabona, 4, 70125, Italy*
[3]*University of Bari, Department of Bioscience , Biotechnology and* Biopharmaceutical*, Via Orabona, 4, 70125, Italy*

Photodynamic Therapy (PDT) is an efficient and alternative treatment for localized tumours. It is based on the combined action of a photosensitizer (PS), visible light, able to match the absorption spectrum of the PS, and endogenous oxygen. The PS, selectively accumulated in malignant tissues, is excited to long-lived excited singlet and/or triplet states by a light with an approprite wavelength. The excited PS, in presence of molecular oxygen, is able to produce Reactive Oxygen Species (ROS) generated by electron transfer (Type I mechanism) and energy transfer (Type II mechanism) reactions. The target tissue is destroyed by ROS which are responsible for cytotoxicity of neoplastic cells and tumor regression since induce cellular damage via apoptosis, necrosis, or both. Among the different classes of compounds examined as potential photosensitizers in PDT, porphyrins and their analogous are considered the most important because they have photochemical and photophysical properties making them potentially suitable for PDT applications. In this research work an amphipathic porphyrins, Chlorophyll *a* (Chl *a*), has been used as PS. The main limitation of the use of Chl *a* was due to its very poor solubility in water and its high tendency to aggregate. In order to solubilize the natural pigments in aqueous solution, Chlorophyll *a*/Cyclodextrins complexes [1] have been studied as supramolecular photosensitizers. Cyclodextrins (CDs) are cyclic oligosaccharides able to encapsulate the PS into their cavity. They have not only optimal drug loading capacities and release properties, but they also are biocompatible with low toxicity. The stability and photodynamic activity of the Chl *a*/CDs systems into aqueous solutions has been primarily studied. Experimental results overall indicated that $^1O_2$, H_2O_2 and $O_2^{\cdot -}$ are the main ROS produced by the studied system [2]. Subsequently Chl *a*/CD systems have been studied in cell culture medium (DMEM) in order to study the Chl *a* aggregation status and the ability of four CDs to solubilize the pigment in the medium for next application *in vitro*. In particular, the interactions between Chl *a* and four CDs, (2-Hydroxypropyl)-β-Cyclodextrin (2-HP-β-CD), (2-Hydroxypropyl)-γ-cyclodextrin (2-HP-γ-CD), Heptakis(2,6-di-*o*-methyl)-β-cyclodextrin (DIMEB) and Heptakis(2,3,6-tri-*o*-methyl)-β-cyclodextrin (TRIMEB) have been estimated by means of absorption and emission spectroscopy [3]. Measurements *in vitro* have been carried out on human adenocarcinoma cells HT-29. Cytotoxicity and photoxicity have been measured using MTT, a test to analyze the cells proliferation, before and after PDT. DIMEB and TRIMEB cyclodextrins have shown high toxicity for HT-29 cells, thus successive studies have been conducted on the Chl *a*/2-HP-β-CD and Chl *a*/2-HP-γ-CD inclusion complexes. The intracellular localization of the PS has been studied by confocal laser scanning microscopy using subcellular organelle markers. The microscope images suggest that the Chl a is localized mainly in lysosomes. ROS level has been determined by DCFDA (2',7'-dichlorofluorescein diacetate). Moreover flow cytometry studies has been carried out using the annexin V and propidium iodide staining in order to determine the cell death mechanism. After PDT, it was found about 50% of cell death and necrosis is the only cell death mechanism involved. In the studied system, apoptosis process is completely absent. The results suggest that Chl *a*/CDs complexes may be a promising candidate for further use in PDT applications.

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Uptake and photo-toxicity of *meta*-tetra(hydroxyphenyl)-chlorin (*m*-THPC), Foslip® and Fospeg® in tumour cell spheroids

Elisa Gaio[1], Francesca Moret[1] and Elena Reddi[3]

University of Padova, Department of Biology, via U.Bassi 58/B, 35121 Padova, Italy, ++39 049 8276335

It is well established that cells grown as monolayers (2D cultures) are far from experiencing the complex environment to which are exposed the cells in solid tumours *in vivo.* It has been repeatedly reported that 2D cultures are not suitable for studying the effectiveness of anti-cancer drugs because the results are not reliable for translation in the *in vivo* setting.[1] A major problem is that in monolayers the cells are exposed to drugs without the interference of physical barriers which create drug concentration gradient and impair diffusion of drugs inside the tumour mass. Non-homogenous drug distribution into the tumour, caused by inefficient diffusion of the drug molecules far from the tumour capillaries, is responsible for poor outcome following chemotherapy. Similarly, non-homogeneous distribution of a photosensitizer (PS) and sub-optimal concentrations in the deep tumour regions are among the major reasons of incomplete responses to photodynamic therapy (PDT). Therefore to improve the PDT efficacy it is imperative to assess the capability of PS of penetrating into the tumour mass and the depth of photo-induced cell damage.

We used multicellular tumour spheroids as 3D *in vitro* tumour models to investigate the diffusibility of the PS mTHPC in the free form (as in Foscan®) and encapsulated in the PEGylated and non PEGylated liposomal formulations, Fospeg® and Foslip®. Confocal microscopy analyses of HeLa multicellular spheroids incubated for 24 h with mTHPC showed a red fluorescence in the external rim of spheroids while the inner core was devoid of any fluorescence indicating very limited diffusion of mTHPC into the cellular mass. The fluorescence images of the equatorial focal plane and the maximum projection images suggest a somewhat deeper penetration into spheroids and more homogeneour distribution of free mTHPC with respect to the liposomal formulations. Independently of the formulation, mTHPC appeared to localise inside the cells while interactions with components of the extracellular matrix were not evident. Irradiation with red light of the spheroids incubated for 24 h with mTHPC caused an arrest of their growth but without significant differences among the three formulations. The viability of the cells (MTS test) in the irradiated spheroids was decreased to about 20% at maximum with Foslip that showed the best efficacy. The LIVE/DEAD test, that discriminates between live and dead cells with, respectively, calcein and ethidium staining, confirmed that a few cells in the inner part of spheroids treated with liposomal mTHPC, were still alive 24 h post irradiation. Detailed analyses of the morphological changes in the irradiated spheroids were carried out by scanning and transmission electron microscopy. While the incubation in the dark with mTHPC did not cause evident morphological changes in spheroids, irradiation caused evident swelling of the cells in the external layers. Liposomal mTHPC, and to a lesser extent free mTHPC, caused more evident destructions of cell-cell interactions causing more easy detachment of the damaged cells from the spheroids indicating some differences in the mechanism of photoinduced damage.

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Silica Coated PbS Nanocrystals with Tunable Emission in the Near Infrared Region conjugated with RGD Peptide for Molecular Targeted Imaging

G. Valente[1,2], N. Depalo[2], M. Corricelli[1], I. de Paola[3], E. Fanizza[1], R. Comparelli[2], M. Striccoli[2], A. Agostiano[1,2], N. Denora[4], V. Laquintana[4], R. M. Iacobazzi[4], M. Saviano[5], A. Del Gatto[3], L. Zaccaro[3], M. L. Curri[2]

[1] Università degli Studi di Bari Aldo Moro, Dipartimento di Chimica, Via Orabona 4, 70125 - Bari, Italy, 0805442027
[2] CNR-IPCF UOS Bari, ViaOrabona 4, 70125 - Bari, Italy, 0805442027
[3] Istituto di Biostrutture e Bioimmagini-CNR, Via Mezzocannone, 16, 80134 - Napoli, Italy, 0812534566
[4] Università degli Studi di Bari Aldo Moro, Dipartimento di Farmacia, ViaOrabona 4, 70125 - Bari, Italy, 0805442767
[5] Istituto di Cristallografia-CNR,Via Amendola 122/O, 70126 Bari, Italy, 0805929148

Near-infrared (NIR) fluorescence imaging is most attractive and rapidly progressing area for early detection, accurate diagnosis, and targeted therapy of various diseases, especially cancer.[1] NIR emitting semiconductor nanocrystals (NCs) are emerging as revolutionary labelling materials for in vivo and deep-tissue imaging of biological targets, due to their high photostability, versatile surface modification and unique tunability in the optical properties.

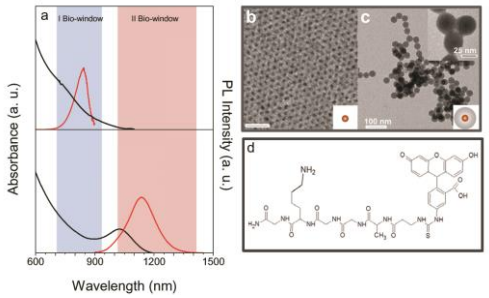

Figure 1. Vis-NIR absorption (black line, a) and PL emission spectra (red line, a) of organic capped PbSNCs with two different sizes. TEM micrograph of PbSNCsbefore (b) and aftergrowth of silicashell (c). Molecular structure of cyclic RGD peptide.

Here, the synthesis of uniform silica coated PbSNCs with emission propertie sconveniently tunable from the first to the second 'biologicalwindow' has been attained.[2] Active targeting has been achieved by coupling the silica coated PbSNCs with a designed cyclic RGD peptide, providing NIR fluorescent nanoprobes able to interact with integrin αvβ3 expressed on the tumorvasculature. [3] The NP/peptide bioconjugates, characterized by a high colloidal stability in physiological media and preservation of the relevant optical properties in the NIR region of electromagnetic spectrum, are promising candidates for targeted NIR molecular labelling and in vivo NIR tissue imaging applications.

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Materials and techniques for photobiology

Plenary lectures and Communications

Single molecule study of prokaryotic gene expression regulation

Matteo Prayer Galletti[1], Alessia Tempestini[1], Carina Monico[2], Lucia Gardini[1], Marco Capitanio[1,3], Renato Fani[4], Francesco S. Pavone[1,3] and Francesco Vanzi[1,4]

[1]*European Laboratory for Nonlinear Spectroscopy (LENS), Via N. Carrara 1, 50019 Sesto Fiorentino (FI), Italy*

[2] *Chemistry Research Laboratory, University of Oxford, 12 Mansfield Road, Oxford, UK*

[3]*Department of Physics, University of Florence, Via Sansone 1, 50019 Sesto Fiorentino (FI), Italy*

[4]*Department of Biology, University of Florence, Via Madonna del Piano 6, 50019 Sesto Fiorentino (FI), Italy*

Prokaryotic cells lack the intracellular compartmentalization typical of eukaryotic cells, where different functions are partitioned in the different organelles. However, several evidences suggest that for example ribosomes[1] are not randomly distributed throughout the cell volume. One key aspect of spatial organization inside bacteria regards the three-dimensional nucleoid organization, the relative position of genes involved in the same metabolic pathway and the mechanism of DNA binding by regulatory proteins. Indeed, bacteria nucleoid has been observed to be dynamic[2] and its spatial reorganization in response to different environmental and metabolic conditions might be expected. Due to the size of bacteria, however, the study of intracellular distributions of proteins and DNA sequences is hindered by the resolution limitations inherent to optical microscopy. Thus, tackling this problem requires a method for the localization of fluorescently labelled molecules with nanometric precision in 3D. We developed a system for localizing and tracking a single fluorescent molecule *in vitro* and *in vivo* with a precision of up to 5-10 nm radially and 10-15 nm axially. We also developed a method for implementing Fluorescence In Situ Hybridization (FISH) in bacteria with single molecule sensitivity, permitting the localization of any chosen DNA sequence. By implementing a two-color detection scheme, this method can be applied to mapping the localization of chosen pairs of genes in different growth conditions. These measurements may allow to check if genes involved in the same metabolic pathway and scattered throughout the chromosome might be spatiallyco-localized in the same "translational" environment when their expression in required.

For the detailed study of the mechanisms of gene expression regulation at the molecular level, on the other hand, we have applied an ultrafast optical tweezer setup for the measurement of target search and operator binding by Lac repressor. Transcription factors and DNA-binding proteins bind their specific target sequences with rates higher than that allowed by 3D diffusion alone. Generally accepted models predict a combination of free 3D diffusion and 1D sliding along non-specific DNA[3]. One important issue in the field of protein-DNA interaction is the understanding of how proteins interact with non-cognate DNA sequences and how they find the sequence of interest along the DNA. We developed a system that permits to detect protein-DNA interaction with sub-ms temporal resolution and nanometer spatial precision[4]. With these measurements we are able to localize protein-DNA interactions along the DNA sequence and measure their dynamics. We find short events (milliseconds), corresponding to interactions with non-specific DNA sequences, and long events (tens of seconds), corresponding to interactions with operator sequences. We used a DNA molecule containing two copies of the LacI O1 (high affinity) and one copy of the O3 (low affinity) operator. The long interactions occur corresponding to the position of the two O1 operators. Short interactions, on the other hand, occur more uniformly along the whole DNA sequence, although with higher probability in correspondence of the operators and in their proximity. Dissociation of both classes of interactions was highly accelerated by an external load. Measurements performed in the presence of IPTG, which mimics the inducer allolactose, resulted in drastically reduced frequency of specific interactions.

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The molecular mechanism of photodimerization in DNA

Roberto Improta

Istituto Biostrutture e Bioimmagini- Consiglio Nazionale delle Ricerche, Via Mezzocannone 16, I-80136 Napoli, Italy

Based on accurate Quantum mechanical calculations on realistic oligonucleotide models in solution, including the effect of the phospho-deoxyribose backbone, and integrating the indication provided by ultra-fast time-resolved optical spectra, we report a thorough description of the main photochemical paths involving a dipyrimidine step within DNA. After describing the excited electronic states responsible for the formation of the most dangerous photoproducts, i.e. cyclobutane dimer (CPD) and 6-4 pyrimidine pyrimidinone adducts (6-4PP), we shall describe the main factors modulating the photoreactivity in DNA, such as nucleotide sequences, duplex conformation, presence of non-standard bases.[1-4] We shall explain why Thymine-Thymine steps are the most reactive sites, why the yield of CPD is much larger than 6-4PP, why 5Methyl-Cytosine is more reactive than Cytosine, how the photochemical paths changes with the excitation wave-length.

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Dual Probe for living cells sensing

Gerardo Abbandonato,[1] Giovanni Signore,[2] Barbara Storti,[1] Fabio Beltram,[1] Ranieri Bizzarri[1,3]

[1]NEST, Scuola Normale Superiore and Istituto Nanoscienze - CNR, Pisa, Italy
[2]Center for Nanotechnology Innovation @NEST, Istituto Italiano di Tecnologia, Pisa, Italy
[3]Istituto di Biofisica – CNR, Pisa, Italy

A fluorescent probe structurally similar to the GFP chromophore is demonstrated to report on the local static dielectric constant (ε). Indeed, the solvatochromic behavior of our sensor was exploited by emission ratiometry to afford the ε values in complex systems (i.e. Triton X-100 micelles, Biotin-Streptavidin interface). The probe showed to be easily bioconjugable and straightforwardly allowed us to obtain the polarity map of several organelles in living cells[1]. Further to emission intensity, fluorescence lifetime revealed to be dependent on the solvent dielectric constant and, at the same time, on the viscosity of the medium according the Förster-Hoffmann[2] equation. We demonstrated that this photophysical behavior is related to the existence of two intermediate excited states, Solvent Relaxed and Twisted Intramolecular Charged State (TICT), whose decay rate constants are governed by the physicochemical parameters of the local nanoenvieronment. This behavior reminds of conventional molecular rotors[3], although the lifetime decays are in this case much longer and allow for efficient lifetime imaging (FLIM) of biological specimens. On account of these properties, the use of our probe as dual sensor of environmental polarity and viscosity by combining emission ratiometry and FLIM will be discussed.

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Unveiling the Spatio-Temporal Organization of TRPV1 Nociceptor in Live Cell Membranes

Barbara Storti[1], Carmine Di Rienzo[1,2], Francesco Cardarelli[2], Ranieri Bizzarri[1] and Fabio Beltram[1,2]

[1]NEST, Scuola Normale Superiore and Istituto Nanoscienze - CNR, Piazza S. Silvestro 12, 56127 Pisa
[2] Center for Nanotechnology Innovation @NEST, Istituto Italiano di Tecnologia, Piazza San Silvestro

Transient Receptor Potential Vanilloid 1 (TRPV1) is a non-selective cation channel that integrates several stimuli into nociception and neurogenic inflammation[1]. Here we investigated the subtle TRPV1 interplay with candidate membrane partners in live cells by a combination of spatio-temporal fluctuation techniques and fluorescence resonance energy transfer (FRET) imaging. My experimental strategies benefited from the use of genetically-encodable fluorescent reporters belonging to the green fluorescent protein family. I targeted the organization of membrane TRPV1 complexes with caveolin-1 and microtubules by combining for the first time FRET with a STICS method based on the extraction of the molecular mean square displacement directly from imaging data (FRET-*i*MSD). We show that TRPV1 is split into three populations with fairly different molecular properties: one binding to caveolin-1 and confined into caveolar structures, one actively guided by microtubules through selective binding, and one which diffuses freely and is not directly implicated in regulating receptor functionality [2]. The emergence of caveolin-1 as a new interactor of TRPV1 evokes caveolar endocytosis as the main desensitization pathway of TRPV1 receptor, while microtubule binding agrees with previous data [3] suggesting the receptor stabilization in functional form by these cytoskeletal components. Our results shed light on the hitherto unknown relationships between spatial organization and TRPV1 function in live-cell membranes.

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Giant vesicles as compartmentalized bio-reactors: optical spectroscopy investigations

Emiliano Altamura[1], Pasquale Stano[2], and Fabio Mavelli[1]

[1]*University "Aldo Moro", Chemistry Department, Via Orabona 4, 70125 -Bari, Italy*
[2] *Science Department, Roma Tre University, Viale G. Marconi 446, I-00146 Rome, Italy*

Giant lipid vesicles (GVs) are widely used as model systems to study lipid and membrane protein behavior, in the hydrophobic domain, and compartmentalized enzyme reactions in the water core. In size and composition, these compartments well mimic the simplified cell environment; for this reason GVs are the perfect candidates for synthetic cell construction [1][2].

One of the most important problem about the GVs preparation is related to the controlled encapsulation of solutes. All the common preparation methods, such as gentile hydration and electroswelling, do not allow a well-controlled entrapment. In 2003 Pautot et al. [3] proposed a successful method for giant vesicle preparation called "droplet transfer method". This method is based on a water in oil macroemulsion and after centrifugation, the droplets are converted in GVs. In this case the water phase of the emulsion contains all the solutes at known concentration.

Thanks to this procedure, a large variety of compounds can be encapsulate such as enzymes [4], membrane proteins with high molecular weight [5], small fluorescent molecules, synthetic highly charged polymers [6], nucleic acids with gene expression kit [7] etc. In this contribution we present the possibility to study these compartmentalized systems as bioreactor that can communicate with an external input giving and internal reaction as output. In the inner aqueous core a simplified metabolic pathway is entrapped able to produce a fluorescent signal when essential substrates, for the cascade reaction, are produced and/or added outside the preformed giant vesicles (**Figure 1**). This process can be monitored by visual inspection (confocal microscopy) for single object analysis or by high-throughput analysis (flow cytometry).

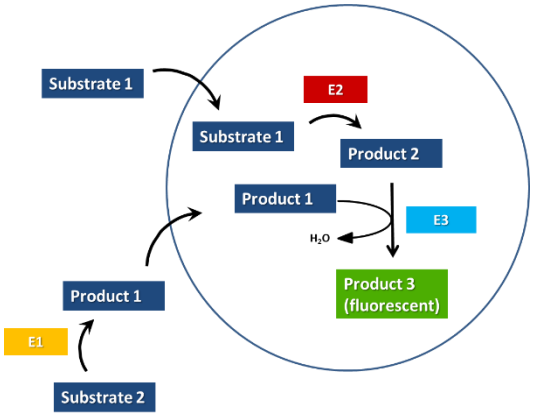

Figure 1 Scheme of cascade reaction in GV: substrate 1 can easily diffuse trough the lipid bilayer, enzyme 1 converts substrate 2 in product 1 that can also permeate the membrane. Once these two species are inside the vesicle, enzyme 2 can produce product 2

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La luce da Caravaggio al contemporaneo

Rosa Anna Pucciarelli

Accademia delle Belle Arti, via Re David, Bari

La luce senza la quale tutto è invisibile. Questo si è tentato e tutt'oggi si cerca di fare nell'arte: riprodurre il visibile, quindi la luce. La relazione "*da Caravaggio al contemporaneo*", illustra con esempi come la luce sia stata ed è oggi rappresentata, più o meno consapevolmente, e come la sua diversa qualità abbia originato differenti poetiche.

Cena in Emmaus, olio su tela, 1601-1602, National Gallery Londra

MALDI-ToF/ToF mass spectrometry analysis of intact bacteriochlorophylls by using 1,5-diaminonaphthalene as electron-transfer secondary reaction matrix

Calvano CD[1,2], Trotta M[3], Italiano F[3], Ventura G[1], Cataldi TRI[1,2], Palmisano F[1,2]

[1]Dipartimento di Chimica & [2]Centro Interdipartimentale di Ricerca S.M.A.R.T. - Università degli Studi di Bari Aldo Moro, Via Orabona 4, 70126 Bari, Italy [3]CNR, Istituto per i Processi Chimico-Fisici, Sezione di Bari, Via Orabona, 4, 70126 Bari, Italy

Photosynthetic organisms are able to convert photons in chemical energy thanks to unique light-harvesting antenna systems occurring inside the cytoplasmic membrane. These antenna complexes are composed of a wide variety of proteins and different chlorophyll pigments acting as binders [1]. Their structures can be divided into chlorophylls (Chls) class found in cyanobacteria and algae up through plants, and into bacteriochlorophylls (BChls), found in phototrophic bacteria [1]. Both pigments consist of a macrocyclic tetrapyrrolic ring system named porphyrin, coordinating a Mg^{2+} ion, and several different side chains, usually including phytol [2]. The main difference between these two types of pigments is related to the saturation state of the porphyrin macrocycle, with bacteriochlorophylls having a much more unsaturated structure than chlorophylls.

Fast identification of BChls and related compounds may be carried out by matrix assisted laser desorption ionization (MALDI) time-of-flight (ToF) MS because of some characteristic advantages as rapid and easy sample preparation, tolerance to salts, and high sensitivity. However, previous analysis of *BChls* showed that demetalation of magnesium porphyrins occurs by using conventional acidic matrices. Indeed pheophitinization (i.e., release of the metal ion) was observed by Persson *et al.*, using a-cyano-4-hydroxycinnamic acid (CHCA) as MALDI matrix, for BChls extracted from *Chlorobium tepidum* green sulfur bacterium that were detected as bacteriopheophytins [3].

Very recently, 1,5-diaminonaphthalene (DAN) was introduced as an electron-transfer secondary reaction matrix for the analysis of chlorophylls [4]. DAN was proved to outperform conventional matrices such as CHCA, dithranol, antracene and even terthiophene, since loss of the metal ion and fragmentation of the phytol–ester linkage are negligible. Here, we report the identification of intact bacteriochlorophylls by MALDI MS in the purple non-sulfur bacterium *Rhodobacter sphaeroides*, a model system for studying both bacteriochlorophyll biosynthesis and assembly of bacterial photosynthetic complexes [5]. These results show the great capability of MALDI MS to follow bacteriochlorophylls biotransformation occurring in different growth conditions of bacteria.

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Nanotechnology-based cancer photodynamic therapy

Elisa Panzarini, Luciana Dini

University of Salento, Department of Biological and Environmental Sciences and Technologies (Di.S.Te.B.A.), via Prov.le Lecce-Monteroni, 73100, Lecce +390832298614

The increasing incidence of cancer have prompted studies to find alternative new treatments and to potentiate conventional ones. The rapid growth of nanotechnology could be deeply exploited in cancer management in terms of diagnosis and therapy.

Among new therapies that can be ameliorate *via* nanotechnology advances, PhotoDynamic Therapy (PDT) appears a promising modality. It is a cancer treatment based on interaction between light, tissue molecular oxygen and PhotoSensitizer (PS) and it has emerged as one of the important therapeutic options in management of cancer over current cancer treatments, due to its advantages, consisting of selective and irreversible destruction of diseased tissue without damaging adjacent healthy ones. However, there are still several technical difficulties in the application of PDT in cancer such as low penetration of light that limits PDT application at superficial lesions; absence of light sources that easy reach body cavities; aggregation of hydrophobic PSs that makes ineffective the parenteral administration.

The NanoMaterials (NMs) allow to circumvent these drawbacks mainly by acting as PSs carriers. Recently, it has been suggested that NMs can also actively participate in photodynamic reaction either by acting as PSs themselves *via* singlet oxygen production under irradiation or as transducers of NIR radiation by emitting wavelength exciting attached PSs. Further, the most recent papers demonstrate the possibility to carry out a double treatment modality, i.e., PDT and chemotherapy, by exploiting photon upconversion process NPs-mediated. Thus, it is easy to forecast that the continue to grow of nanotechnology and its application to cancer PDT will potentiate this treatment that will become ever more convincing.

Near Infrared emitting PbS-lipid nanocarrier for bioimaging applications

Vincenzo De Leo[a], Nicoletta Depalo[b], Michela Corricelli[a,b], Roberto Comparelli[b], Roberto Gristina[c], Marinella Striccoli[b], Lucia Catucci[a], Lucia Curri[b], , Gianpiero Valente[a], Eliana Casamassima[a], Giorgio Caliandro[a], Angela Agostiano[a,b]

[a]*Dipartimento di Chimica, Università di Bari, Bari, 70125, Italy*
[b]*CNR-IPCF, Consiglio Nazionale delle Ricerche, Istituto per i Processi Chimico-Fisici, Bari,70125, Italy*
[c]*CNR-IMIP, Consiglio Nazionale delle ricerche, Istituto di metodologie inorganiche e dei plasmi, Bari, 70125, Italy*

Near infrared (NIR) fluorescent probes are highly suitable for imaging of biological tissues, that show very low absorption and auto-fluorescence in the so-called first (650-950 nm) and second (1000-1350 nm) biological NIR windows. In these regions therefore, a maximum penetration of radiation in the tissues can be achieved [1]. The first NIR transparency window is defined by a local minimum in the absorption spectrum of biological tissue [2]. However not only absorption but also scattering events can attenuate photons emitted from a source embedded in turbid media, such as tissue. The effect of scattering on the effective penetration depth of tissue can be minimized at wavelengths ranging in the second NIR window. PbS nanocrystals (NCs), are a promising candidates for imaging in this region, in a framework characterized by a dearth of available fluorophores. However, the potential toxicity of lead based NCs requires to be suppressed with proper surface modification, as recently showed [3]. Aim of this study was to functionalize the hydrophobic PbS NCs with biocompatible phospholipids, embedding them in microeterogeneus systems, more specifically in the core of micelles and in the bilayer of liposomes. Hydrophobic, colloidal PbS NCs were synthesized with oleic acid (OA) as capping agent. Replacing the original capping ligand onto NCs with dodecanethiol (DDT), as confirmed by FT-IR spectra, a shift of the emission signal from the first to the second biological NIR window was determined. Such shift can be probably attributable to NC ripening, likely ascribable to an oriented attachment mechanism. Micelle-to-Vesicle transition method was employed to introduce DDT capped PbS NCs within the phospholipid bilayer of liposomes. DLS investigations revealed that the mean diameter of the obtained liposomes was about 192±90 nm. TEM images showed entirely loaded liposomes with nanocrystals well dispersed in the lipid domain of the vesicles. Photoluminescence properties of PbS-liposomes were similar to those of the pristine PbS. DDT capped PbS NCs was successfully embedded also in PEG-modified phospholipid micelle, as demonstrated by the TEM images. DLS investigations revealed that the mean diameter of the micelles obtained was about 148±45 nm. The spectroscopic characteristics of PbS were retained after encapsulation in micelles, as shown by photoluminescence spectra. In vitro investigation was also performed by using Saos-2 cells to assess cytotoxicity of PbS NCs after incorporation in micelles or in liposomes. The Blue Coomassie test allowed to identify the cellular morphology and to check for any changes caused by the contact with the samples. The number of cells and the morphology of individual cells were unchanged up to a concentration of 8 mM in PbS. Beyond this value, the cell morphology continues to remain unchanged but decreases the number of cells. Cell viability was evaluated by MTT assay at 24 h and 48 h. Cell viability in the presence of liposomes and micelles containing PbS was maintained above 60% up to a concentration of 2 mM and decreased progressively with increasing concentration of PbS. In the case of liposomes, however, the vitality was maintained higher compared to micelles. Confocal microscopy was used to demonstrate the cellular uptake in Saos-2 cells. The images revealed a significant uptake of PbS loaded nanocarriers, suggesting that the proposed phospholipid functionalization of PbS NCs represent a significant strategy for in vitro and in vivo imaging.

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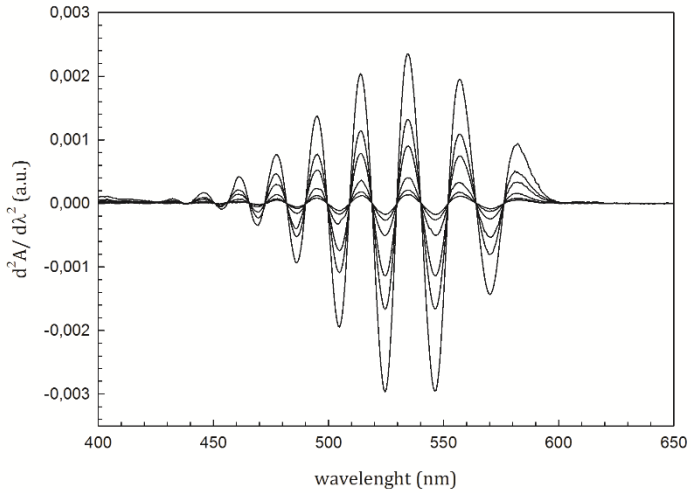
Posters

Heavy metal ions effect on light-harvesting complexes of *Rhodobacter sphaeroides* studied by derivative spectroscopy

Simona la Gatta[1], Alessandra Antonucci[1], Francesco Milano[2], Francesca Italiano[1], Massimo Trotta[2]

[1] Università degli Studi di Bari Aldo Moro, Dipartimento di Chimica, Via Orabona 4, 70125 - Bari, Italy, 0805442027
[2] Istituto per i Processi Chimico Fisici-CNR, Bari, Via Orabona 4, 70125 - Bari, Italy, 0805442027

The non-sulfur, purple, facultatively phototrophic bacterium *Rhodobacter* (*R.*) *sphaeroides* represents a unique model for the investigation of the structure, function and biosynthesis of the energy-transducing system in photosynthetic machineries. Photosynthetic units share a basic architecture, composed by an efficient light collecting system, which funnels light to the reaction center, where photons are converted into chemical potential energy with a quantum yield close to unity. In the *R. sphaeroides* wild type (strain 2.4.1), two distinct antenna complexes are present: the LH-II, absorbing at 800 and 850 nm, and the LH-I, with a maximum at 870 nm, appearing as a shoulder of the 850 nm LH-II band[1]. Strong dependence of the absorption spectrum on changing growing conditions, i.e light intensity or heavy metal ions concentration, requires to find out a robust, non-destructive instrumental method of investigation which could help to quickly solve the complex structure of the absorption signals in the presence of different stress factors. In this work, we present second-order derivative spectroscopy as the ideal technique to tackle this issue. Peaks that originate from derivation correspond to a maximum in the original spectrum, but with the advantage of a sharpening effect, which enables the precise assessment of relevant wavelengths[2,3]. Such effect was successfully tested on potassium permanganate solutions, whose composite band, presenting seven overlapped peaks located in the visible region of the electromagnetic spectrum, was separated in well-distinct signals, even at very low concentrations (Figure). Interestingly, Lambert-Beer Law is maintained, allowing an accurate calculation of peak ratios directly on the sharpened second derivative spectrum. This great advantage was exploited to investigate the effect of the presence of heavy metal ions on the LH complexes within cultures of *R. sphaeroides* 2.4.1 cells. This was possible through the precise individuation of absorption maxima and the evaluation of relative ratios between the three LH-I and LH-II peaks. Preliminary results clearly show a direct influence of tested metals on the biosynthesis of the LH-I complex, thus confirming the potentialities of the proposed technique as a promising tool for the evaluation of the chronic effect of exposure to pollutants during bacterial growth.



Control test of second-derivative spectroscopy on seven potassium permanganate solutions

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Nanoscale protein diffusion by STED-based spatiotemporal fluorescence correlation spectroscopy

Paolo Bianchini[1], Francesco Cardarelli[2], Mariagrazia di Luca[3], Alberto Diaspro[1] and Ranieri Bizzarri[1,3]

[1]Nanophysics, IIT—Italian Institute of Technology, Genoa, Italy
[2]Center for Nanotechnology Innovation @NEST, Istituto Italiano di Tecnologia, Pisa, Italy
[3]NEST, Scuola Normale Superiore and Istituto Nanoscienze - CNR, Pisa, Italy

Fluorescence Correlation Spectroscopy (FCS) represents an established technique to recover single-molecule diffusion and binding properties in cells. Recently, scanning microscopy imaging was applied to add a spatial dimension to the classic, purely temporal, FCS modality: spatiotemporal FCS (stFCS) provides details about the routes that are followed by the diffusing particles or molecules in the specimen [1]. We report on the combination of spatiotemporal fluorescence correlation spectroscopy (stFCS) and stimulated emission depletion (STED) to monitor intracellular protein diffusion at spatial resolution below the optical diffraction limit (superresolution). Our method was validated both *in vitro* and at intracellular level by following the diffusion of fluorescent nanocapsids and of GFP bound to SV40 Nuclear Localization Signal (NLS), respectively. NLS-GFP represents a well-known model of actively nuclear-imported protein that has been the subject of intense research by some of us [2]. The relevance of our approach was demonstrated by the discovery of the persistence of complexes between nucleocytoplasmic transporters and NLS-GFP at distances >500 nm from the nuclear envelope, a phenomenon otherwise invisible at the best resolution of conventional confocal imaging mode . We should stress that, in principle, the resolution of stFCS diffusional maps is limited only by the photophysics of the fluorescent reporter in STED conditions [3].

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Laurdan monitors different lipids content in Eukaryotic membrane during embryonic neural development

Gabriele Bonaventura[1], Maria Luisa Barcellona[2], Ottavia Golfetto and Enrico Gratton[3]

[1]*University of Catania, Department of Drugs Sciences, Viale Andrea Doria 6, 95129 Catania, Italy, +39 095/7384064*
[2]*Laboratory for Fluorescence Dynamics, Department of Biomedical Engineering, University of California, Irvine, California*

Membrane fluidity changes are implicated in a range of biological processes including signaling, membrane fusion, endocytosis, and many others [1]. Although the role of membrane fluidity during development has been widely discussed [1–3], a systematic study of changes in membrane fluidity during embryo development has not been carried out. Lipids and lipid domains play a fundamental role in the structural organization of the plasmatic membrane of eukaryotic cells. Lipids in biological membranes are fundamental for the boundary functions of cells, including stimuli to growth and to immunological and stress response, delivered from the environment to the cell interior. Membranes of internal organelles allow the compartmentalization of cell functions.

The complexity of the membrane lipid composition has suggested the coexistence of domains characterized by different dynamical properties in the membrane plane as sites for a putative preferential partitioning of proteins and solutes, for modulating membrane activity and for diffusion along the plane and through the bilayer [4-5].

We describe a method based on fluorescence-lifetime imaging microscopy (FLIM) to assess the fluidity of internal and external membranes (nuclear and cytoplasmic) in neuronal cells at different stages of pre-natal development (mNPSCs) [day 12 (E12) and day 16 (E16) of gestation]. For the FLIM measurements, we chosen the Laurdan probe which is commonly used to evaluate water penetration in model and in natural biological membranes. Using the FLIM approach, we built a fluidity scale based on calibration with model systems of different lipid compositions. In neuronal cells, we found a marked difference in fluidity between the internal membranes and the plasma membrane, being the plasma membrane the less fluid. However, we found no significant differences between the two cell groups, E12 and E16. A comparison with NIH3T3 cells, a mouse embryonic fibroblast cell line, has showed that the plasma membrane~~s~~ of E12 and E16 cells is significantly more fluid than the plasma membrane of the embryonic fibroblast cells, characterized by a defined competence devoted to a structural function, proof of the early supra-molecular organization, at this stage of development, of neural precursors cell membranes.

This result is justified by the highly defined competence of the fibroblasts devoted to a structural function, exemplified by a branched cytoplasm surrounding an elliptical, speckled nucleus having two or more nucleoli.

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Effect of luminescent nanocrystals containing phospholipids micelles on primary coltures of rat astrocytes

Tiziana Latronico[a], Nicoletta Depalo[b], Gianpiero Valente[b,c], Matilda Colella[a], Elisabetta Fanizza[a], Anna Fasano[a], Valentino Laquintana[d], Nunzio Denora[d], Marinella Striccoli[b], Angela Agostiano[c], Grazia Maria Liuzzi[a], M. Lucia Curri[b]

[a]*Università degli Studi di Bari Aldo Moro, Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica, Via Orabona 4, 70125 - Bari, Italy*

[b]*CNR-IPCF, Consiglio Nazionale delle Ricerche, Istituto per i Processi Chimico-Fisici, Bari,70125, Italy*

[c]*Dipartimento di Chimica, Università di Bari, Bari, 70125, Italy*

[d]*Università degli Studi di Bari Aldo Moro, Dipartimento di Farmacia – Scienze del Farmaco, Via Orabona 4, 70125 - Bari, Italy*

Semiconductor fluorescent colloidal nanocrystals (NCs) present a significant potential in cellular imaging and medical diagnosis, thus offering a powerful tool for specific application in neuroscience, for diagnosis of neurological disorders. Luminescent NCs display unique and superior optical properties compared with traditional organic fluorescent dyes, such as broadband excitation, narrow bandwidth emission, high quantum yield, resistance to quenching and good photochemical stability. In addition, due to their small size, fluorescent NCs can be suitably designed to interact with neuronal and glial cells of Central Nervous System (CNS), at cellular and subcellular levels, thus representing good candidates for tracking studies of molecular dynamics of intra or intercellular process [1]. Properly functionalized and bioconjugated NC-based systems may further provide powerful nanoplatforms for investigating the effects of drugs or other biologically relevant molecules for the care and treatment of neurological diseases [2,3]. Despite the growing literature on the use of luminescent NCs as imaging and diagnosis agents by using a wide variety of cell types, typically tumor cells or immortalized cells, only a limited number of studies have so far explored their applications in primary neuronsand glial cells. Therefore, approaches for an early and sensitive detection of glia and neuron responsiveness to luminescent NCs are necessary.

Astrocytes are the major glial cell type in the brain and their activation is one of the key components of the cellular responses to stress and brain injuries. Thus astrocytes may represent a useful target to study the interaction between NCs and CNS cells. Indeed, understanding and evaluation of the potential cytotoxicity of colloidal NCs on in vitro systems is a fundamental pre-requisite for their use *in vivo* in clinical studies.

In this work, a comprehensive and systematic investigation on the *in vitro* toxicological effect of Cadmium Selenide based luminescent NCs on primary cultures of rat astrocytes has been performed. Cytotoxicity response of empty micelles based on polyethylene glycol (PEG) modified phospholipids has been compared to that of those containing NCs [4], in order to investigate the effect on cell viability of both inorganic NC and organic coating molecules employed to protect NC surface. Furthermore, since the surface charge and terminal groups influence the cell interaction and toxicity, PEG-modified phospholipid micelles without terminal groups and with two different functional groups, namely amine and carboxyl groups, have been tested.

The ability of PEG-lipid micelles to be internalized into the cells has been qualitatively and quantitatively assessed by fluorescence microscopy and photoluminescence (PL) assay. Interestingly, the results clearly indicated that a very low concentration of NC containing micelles is required to be properly imaged within the cells. Finally, the study has allowed to define a suitable experimental procedure, by optimizing the relevant parameters, for application of the luminescent colloidal NCs as convenient optical probe also for future *in vivo* experiments.

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Photodynamic Therapy applications based on Chlorophyll-a/Alginate Microparticles

Paola Semeraro[1], Paola Fini[2], Maria Francesca Gargano[1], Tiziana Placido[1], Vito Rizzi[1], Angela Agostiano[1,2] Pinalysa Cosma[1,2]

[1] Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", Via Orabona 4,70126, Bari, Italia
[2] Dipartimento di Chimica, Consiglio Nazionale delle Ricerche CNR-IPCF, UOS Bari, Via Orabona 4, 70126 Bari, Italia

The aim of this work is the study and the physico-chemical characterization of a drug delivery system functionalized with Chlorophyll-a (Chl-a), acting as photosensitizer (PS), for Photodynamic Therapy (PDT) [1] applications. Chl-a, a natural photosynthetic pigment, entrapped in an aqueous matrix made of Ca^{2+} cross-linked alginic acid [2] keeps its characteristic photosensitising properties usable in PDT. After administration, the PS accumulated in malignant tissues produces reactive oxygen species (ROS), mainly singlet oxygen ($^1O_2$), upon light irradiation. This creates an oxidative stress state, responsible for tumor regression, since it induces cellular damage via apoptosis, necrosis, or both. Chl-*a*/alginate microspheres were characterized by means of different techniques as UV-Vis absorption and emission spectroscopy, FT-IR spectroscopy, Atomic Force Microscopy, Dynamic Light Scattering and Differential Scanning Calorimetry. Moreover, it was estimated ROS production by means of a selective luminescent probe, Singlet Oxygen Sensor Green [3]. There are ongoing *in vitro* tests on human adenocarcinoma cell line (HT29).

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Eco-toxicological evaluation of silver nanoparticles by using the bacterium *Rhodobacter sphaeroides*

Francesca Italiano[1], Vincenzo De Leo[1], Davide Avolio[1], Angela Agostiano[1-2], Lucia Catucci[1], Roberto Comparelli[2], Massimo Trotta[2]

[1] Università degli Studi di Bari "Aldo Moro", Dipartimento di Chimica, via E. Orabona, 4 – 70126 Bari, Italy
[3] CNR - Istituto per i Processi Chimico-Fisici, Sezione di Bari, via E. Orabona, 4 – 70126 Bari, Italy

Rhodobacter sphaeroides is a facultative phototrophic anoxygenic proteobacterium with promising applications in bioremediation [1]. AgNPs are being increasingly used since the past decade in consumer goods, mainly for their antimicrobial properties [2]. Ecological concerns are hence increasing on AgNPs release into the environment. European civil servants and policy-makers European are making efforts in implementing the REACH regulation to nanomaterials, aiming to assess their potential adverse effects on health and environment by using alternative methods, that exclude the use of vertebrates. *R. sphaeroides* is a promising model system in nanoparticle ecotoxicity evaluation and its potential applications in nanobioremediation is a plausible scenario.
The exposure experiments were conducted *via* two different assays: exposing bacterial cells to AgNPs growth inhibition assay in liquid broth and on agar gel. The effect of particle dimension (10 – 160 nm) and concentration (0 – 2 mg/L) on bacterial growth was evaluated. Dose-response curves were obtained for each investigated AgNP dimension and the corresponding inhibitory factors EC_{50} were determined. Bacterial sensitivity to AgNPs was found to be dependent on the assay. The observed results could be probably related to the different release entity of Ag^{+} ions and to the aggregation/agglomeration state of nanoparticles in different growth media.

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Bacterial phototrophic biomass as biosorbent for the removal of Nickel(II) from waste-waters

Desiree Dattis[1], Maria Rachele Guascito[1], Simona Rella[1], Daniela Chirizzi[1,2], Disma Mastrogiacomo[1], Livia Giotta[1], Francesca Italiano[2], Massimo Trotta[2], Francesco Milano[2], and Giorgio Martina[1]

[1] *University of Salento, Department of Biological and Environmental Sciences and Technologies (DiSTeBA), S.P. Lecce-Monteroni, 73100 Lecce, Italy*

[2] *University of Salento, Department of Cultural Heritage - Via Birago 7, 73100 Lecce, Italy.*

[3] *IPCF-CNR, Sez. Bari, via Orabona 4, 70126 Bari, Italy*

The intensification of industrial technology increased heavy metal contamination in aquatic systems. Since inorganic pollutants cannot be degraded, an efficient removal system must be designed in order to detoxify heavy metal-contaminated wastewaters. Metal ion biosorption by microorganisms is an interesting mechanism which can be exploited for this purpose.

The purple bacterium *Rhodobacter sphaeroides* is known for its ability to tolerate under phototrophic conditions high concentrations of several heavy metal ions and to bioaccumulate Ni^{2+} and Co^{2+} ions[1].

In this work Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy and X-Ray Photoelectron Spectroscopy (XPS) have been employed for getting information about Ni^{2+} binding onto *R. sphaeroides* cell surface.

The ability to bind nickel ions was evaluated both in free cells and in calcium alginate-immobilized biomass. Before Ni^{2+} exposure the bacterial biomass was washed thoroughly with KCl 0.1 M in order to fully saturate with K^+ ions the negatively charged cell envelopes. XPS measurements revealed that treatment with Ni^{2+} resulted in full displacement of K^+ ions from free *R. sphaeroides* cells, indicating high affinity between nickel ions and surface functional groups. Moreover ATR-FTIR measurements showed that Ni^{2+}-treatment induce the shift of absorption bands arising from symmetric and asymmetric stretching modes of cell surface carboxylate groups, in agreement with their involvement in metal complexation.

Calcium alginate beads entrapping bacterial biomass were prepared dropping a cell suspension supplemented with sodium alginate into 2% $CaCl_2$. XPS analysis of Ni^{2+}-treated beads revealed that the exposure of cells to Ca^{2+} strongly inhibited Ni^{2+} uptake suggesting that displacement of Ca^{2+} by nickel ions does not occur.

These data are of interest in order to identify optimal conditions for the efficient removal of Ni^{2+} by means of phototrophic bacterial biomass.

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Bacterial phototrophic biomass as a bio-catalyst for the reduction of Chromium(VI) in waste-waters

Desiree Dattis[1], Disma Mastrogiacomo[1], Angela Maria Metrangolo[2], Livia Giotta[1], Francesca Italiano[3], Massimo Trotta[3], Francesco Milano[3], and Gabriele Rinelli[1]

[1]University of Salento, Department of Biological and Environmental Sciences and Technologies, DiSTeBA, S.P. Lecce-Monteroni,73100 Lecce, Italy [2] ISPA-CNR, Sez. Lecce, S.P. Lecce-Monteroni,73100 Lecce, Italy
[3] IPCF-CNR, Sez. Bari, via Orabona 4, 70126 Bari, Italy

Hexavalent chromium represents an outstanding risk for the environment and the health of human beings, as it is considerably involved in the genesis of cancer and other fatal diseases. Biological reduction of Cr(VI) to Cr(III) is a potentially useful mechanism to remediate chromium (VI) pollution and to detoxify contaminated wastes.

The photosynthetic purple bacterium *Rhodobacter sphaeroides* is known for its ability to tolerate high concentrations of several heavy metal ions, to bioaccumulate nickel and cobalt, and to reduce oxyanions as tellurite, selenite and chromate. The response of the carotenoidless mutant R26 to chromate stress under phototrophic conditions has been recently investigated by biochemical and spectroscopic measurements, proteomic analysis and cell imaging, revealing good Cr(VI) reduction ability associated with morphological and compositional changes of the cell envelope, while no specific stress-induced chromate-reductase activity was found in the soluble proteome[1].

Phototrophic biomass of *Rhodobacter sphaeroides* strain R26, harvested, washed, and stored at -20°C, was just thawed and used as Cr(VI) reduction catalyst. Chromate solutions, buffered at neutral pH and supplemented with a mixture of succinate, malonate and glucose as electron donors, have been employed for simulating the waste-water environment. The decrease of Cr(VI) concentration triggered by cells addition was evaluated by the diphenylcarbazide (DPC) assay. The analysis of reaction kinetics revealed that *Rhodobacter sphaeroides* resting biomass acts as an excellent bio-catalyst promoting chromate reduction by oxidizable carbon compounds. The role of abiotic variables such as pH, light, temperature and oxygen concentration was also assessed. Our data extend the information available about this phototrophic microorganism and elucidate its potential in Cr(VI) bioremediation applications.

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Folate- tailored amphiphilic cyclodextrins as carriers of pheophorbide for targeted PDT

Giuseppe Sortino[1], Anna Piperno[2], Angela Scala[2], Valentina Rapozzi[3] and Antonino Mazzaglia[1]
[1]*CNR-ISMN c/o Dip. Scienze Chimiche dell'Università di Messina, Viale Ferdinando Stagno D'Alcontres 31, 98166, Messina, Italy*
[2]*Dip. Scienze Chimiche, Università di Messina, Viale Ferdinando Stagno D'Alcontres 31, 98166, Messina, Italy*
[3]*Department of Medical and Biological Sciences, University of Udine, P.le Kolbe 4, 33100 Udine, Italy*

Over the past two decades, scientists focused their interests on design of novel nanotherapeutic tools, prompt to actively deliver drugs in tumor tissue. One of the most used strategies relies on the functionalization of carrier system with receptor targeting groups such as folate, antibody, saccharides and peptides. Folate receptor-α (FR-α) is over-expressed in different cancer cell lines, thus the modification of nanosystem with folate group, represents a well-established strategy for tumor targeting. Cyclodextrins (CyDs), a well-known class of macrocyclic carriers, have been modified with folate group to deliver anticancer drug in (FR-α) cell positive [1]. Here, we exploit the host–guest interaction of non ionic amphiphilic cyclodextrins (ACyDs, SC6OH) [2] with a folate–adamantanyl derivative (Ada-Fol) to design a novel tailored drug delivery system. Ada-Fol was newly synthesized by coupling of the adamantanyl-carboxylate and the γ-carboxylic group of folic acid to a diamine spacer, and characterized by ^{1}H-NMR and MALDI-MS. Nanoassemblies of SC6OH@Ada-Fol loaded with Pheophorbide (Pheo) [3], a photosensitiser with high PDT efficacy were produced and fully characterized.

SC6OH@Ada-Fol system has been prepared by adding PBS (10 mM, pH 7.4) to a mixed (organic) film of SC6OH and Ada-Fol at 2.5:1 SC6OH/Ada-Fol molar ratio. This dispersion has been used to dissolve Pheo at 2.5:1:1 SC6OH:Ada-Fol:Pheo molar ratio. Pheo-loaded SC6OH@Ada-Fol nanoassemblies were investigated by complementary techniques such as UV-Vis, steady-state fluorescence and characterized to elucidate size, drug loading and to get insight on the sites of entrapped photosensitizer interaction. In order to verify the biological properties, we have begun to evaluate *in vitro* the effectiveness of SC6OH@Ada-Fol/Pheo on cell growth on different breast cancer cell lines (MCF-7, MD-231). Preliminary data indicate that the nanoassemblies, upon light irradiation, inhibits cell proliferation depending on the expression of folate receptor. Additional experiments are underway.

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Photostability of drugs of abuse in hair irradiated in a solar box

Marianna Tucci[1], Donata Favretto[1], Santo Davide Ferrara[1], Giorgia Miolo[2]

[1]Forensic Toxicology and Antidoping, University-Hospital of Padova (Italy)
[2]Department of Pharmaceutical and Pharmacological Sciences,University of Padova, ITALY

The concentration of drugs in hair may be altered by physical and chemical factors, among which the use of cosmetics or chemical treatments such as dying perming or bleaching, and environmental exposure. Solar light was demonstrated to alter molecular structures of drugs when irradiated in solutions or in biological fluids, decreasing the concentrations of drugs and/or producing new compounds/metabolites. Studies were performed for different classes of compounds by exposure of hair to sun (cannabinoids) or controlled UVA and UVB (cocaine, opiates, methadone) .

In order to better understand the role and mechanisms of solar light exposure in decreasing hair concentrations of drugs, and following our previous photodegradation studies on UVA and UVB induced changes (*Drug Test Anal* 2014 , **6**, 78-84), we undertook the present work irradiating true positive hair samples, containing cocaine and metabolites, in a solar box, reproducing the whole spectrum of sunlight.

Authentic positive hair was selected from samples routinely tested at the laboratory that had previously tested positive for cocaine. Irradiation was performed in a Suntest CPS+ (Atlas, Linsengericht, Germany) equipped with a 1.8 kW xenon lamp and a glass filter (cut off 310 nm) according to Option 1 of ICH Guideline Q1B (1998). 25 hair samples were collected of different natural colours (blond, brown, dark brown, black). Hairs, 5–7 cm long, were divided into two approximately identical strands: the former was put between two 5 x 5 cm optical glasses and exposed at 765 W/m^2 (310-800 nm) for 48 hours to an endpoint corresponding to two months exposure under the sunlight, and the latter was kept as a dark control in the same chamber of irradiation covered with an aluminium foil.

Hair samples were washed and 10 mg were extracted and analyzed by a validated method already proposed by Favretto et al. (*Drug Test Anal* 2014 , **6**, 78-84) encompassing micropulverized extraction and liquid chromatography-high accuracy, high resolution mass spectrometry (HPLC-HRMS) detection on an LTQ-Orbitrap (Thermo Fisher Scientific, Bremen, Germany). The % photodegradation was calculated as [100 *(drug concentration in the dark - drug concentration after exposure)/drug concentration in the dark].

The concentration ranges in the intact samples were 0,16 – 40,0 ng/mg and 0,05 - 19 ng/mg respectively for COC and BZE. 69 % of samples exhibited a decrease of COC concentration in post-irradiation samples, with percent reduction from to 6 % to 72 % (mean 37,8 %); in 23 % of samples BZE decreased from 10 to 50 % of its initial concentration; in 46 % of samples BZE increased from 6 to 23 %; in 31 % of samples both COC and BZE contents did not vary. BZE increase was observed only in samples that exhibited COC decrease, suggesting that photodegradation of the parent compound generates BZE that remains incorporated into the hair shaft. If this holds true, for the 6 samples (23 %) that exhibited both COC and BZE decrease, the further degradation of BZE originally formed by photodecomposition of COC can be envisaged. No relation could be found with hair color or hair thickness. The possible contribution of hair damage is under investigation by imaging techniques. Results will be presented also for nor-cocaine and cocaethylene in a smaller group of samples.

When compared with our previous studies, when only specific UV components of sunlight (UVB lamp, emitting irradiation with a *peak at* 311–*312* nm and UVA lamp, peaked at 365 nm) were used for irradiation, experiments in the solar box evidenced a similar percent of “degraded” hair samples (69 % solar box *vs* 62 % UVA/UVB) but a higher photodegradation yield of COC (mean 37 % *vs* mean 10 % respectively). The increase of concentration of a metabolite upon concomitant degradation of its parent compound highlights the peculiar role of whole sunlight and prompts for further studies, including other classes of compounds.

In vivo non-invasive evaluation of actinic keratosis response to MAL-PDT by reflectance confocal microscopy

Venturini Marina, Zanca Arianna, Sala Raffaella and Calzavara-Pinton Piergiacomo

University of Brescia, Department of Dermatology, Spedali Civili di Brescia, p.le Spedali Civili, 1 – 25123 Brescia, Italy

Introduction: Photodynamic therapy (PDT) with methyl-aminolevulinate (MAL) is an approved non-invasive treatment option for actinic keratoses (AKs). In vivo reflectance confocal microscopy (RCM) is a non-invasive tool for real-time imaging of epidermis and superficial dermis in vivo, generating horizontal skin sections at high resolution close to conventional histology. RCM has been previously reported to facilitate the in vivo evaluation of several pigmented and non-pigmented skin lesions, including AKs.

Methodology: The aim of this study was to investigate the use of in vivo RCM in evaluating AK response to MAL-PDT. For this reason a total of 10 biopsy-proven AKs in 10 outpatients (2 females and 8 males, age ranging between 67 and 82 years) were treated. Patients presented AKs located on the face (2 AKs) or head (8 AKs). MAL-PDT was performed with a MAL cream (160 mg/g), applied for 3 hours under an occlusive and opaque dressing prior to illumination from a LED source (wavelength range: 635±18 nm; light dose: 37 J/cm2). All lesions received two treatment sessions 7 days apart. RCM investigation was performed before and after PDT with a Vivascope 1500® (Lucid Technologies, Henrietta, NY, U.S.A.) microscopy. Confocal imaging criteria for in vivo diagnosis of AK include presence of hyperkeratosis, parakeratosis, keratinocyte atypia, nuclear and cellular pleomorphism, architectural disarray, inflammatory cells, blood vessel dilatation and solar elastosis. RCM-guided punch biopsies was taken at 3 months in all patients for histopathological examination of the treated area.

Result and Conclusions: At 3 months follow-up, complete clinical response was observed by clinical examination in 9 out of 10 lesions and a partial clinical response in 1 lesion. In vivo RCM evaluation identified complete response in 7 lesions and partial response in 3 out of 10 lesions, detecting 2 residual AKs in subclinical form, missed by clinical examination. Histological analysis confirmed these results. Although histopathology remains the “gold standard” for diagnosis of AKs, repeated biopsies may not always represent a practicable approach to the diagnosis and management of these lesions, because it is expensive, time-consuming and associated with scar formation. In vivo RCM may be a new alternative tool for the non-invasive diagnosis of AKs and evaluation of AK response to non-invasive treatments, as MAL-PDT. In addition in vivo RCM can improve the ability of dermatologists to diagnose AKs providing higher diagnostic accuracy than clinical evaluation and to detect subclinical persistent AKs after MAL-PDT. This approach has two main advantages: early treatment of relapses at the subclinical state and preservation of good cosmetic outcomes obtained with MAL-PDT, without the disadvantages of repeated invasive skin biopsy and conventional histology.

Indice dei nomi

Comitato Organizzatore

Alessandra Antonucci
Lucia Catucci
Vincenzo De Leo
Francesca Italiano
Francesco Milano
Massimo Trotta

Comitato Scientifico

Gianfranco Canti
Enrico Caruso
Giovanni Checcucci
Giorgia Miolo
Valentina Rapozzi
Giovanni Romano
Elena Reddi
Massimo Trotta
Marina Venturini

Segreteria Organizzativa

Alessandra Antonucci
Istituto per i Processi Chimico-Fisici – CNR sede di Bari
Via Orabona, 4 – 70126 Bari