PHOTOBIOLOGY 2010

3rd joint meeting of the French and the Italian Societies for Photobiology

PARIS

October 25-26, 2010

3rd joint meeting of the French and the Italian Societies for Photobiology

PHOTOBIOLOGY 2010

FIAP Jean Monnet, PARIS

25-26 October, 2010

The colloque is sponsored by:

- CNRS (Institut de Chimie)
- Ville de Paris
- L'Oréal
- Galderma
- European Society for Photobiology

Foreword

The italian and french photobiologists have a long history of collaboration and friendship. The two sister societies, the « Società Italiana di FotoBiologia » and the « Société Française de Photobiologie » (SFPb), have sealed this friendship through the organization of two successful and memorable joint meetings, PHOTOBIOLOGY 2000 in Aix-les Bains and PHOTOBIOLOGY 2004 in Pisa. Today, the Italian and the French Societies for Photobiology are pleased to welcome you in Paris to their third joint meeting, PHOTOBIOLOGY 2010.

As previously, the program is divided into 4 broad topics that reflect the diversity and the richness of the research in both countries. Communications by young scientists are encouraged to ensure a future to our tradition and to strengthen our history of collaboration and friendship.

We wish you a successful and enjoyable meeting in Paris.

Evelyne Sage
President of French Society for Photobiology

and

Gianfranco Canti
President of the Italian Society for Photobiology

LOCATION OF THE MEETING:

FIAP Jean Monnet

30 rue Cabanis, 75014 PARIS

Metro/RER: Saint Jacques, Glacière (line 6), Denfert-Rochereau (RER B, lines 4 and 6)

http://www.fiap.asso.fr/

PROGRAM

Monday 25 October

8h30 - 8h55: Registration

8h55 - 9h00: Welcome address

Morning symposium: ***Molecular targets in photobiological processes – nanotechnology***

Chairpersons: Franco Fusi and Jean Cadet

9h00 - 9h30: Thierry Douki (CEA Grenoble)
"Pyrimidine cyclobutane dimers in DNA : do we know everything after 50 years ?"

9h30 - 10h00: Roberto Pini (Firenze)
"The potential of metal nanoparticles as new nanochromophores for diagnostics, imaging and therapy"

10h00 - 10h30: Coffee break

10h30 - 10h50: Paola Taroni (Politecnico Milano)
"Fluorescence of fullerol in human lens and retinal pigment epithelial cells"

10h50 - 11h10: Thomas Gustavsson (CEA, Saclay)
"Ultrafast fluorescence spectroscopy studies of electronically excited states in DNA"

11h10 - 11h30: Benoit Dubertret (ESPCI, Paris)
"Quantum Dots nanoparticles for cancer detection"

11h30 - 11h50: Monica Camerin (Padova)
"The *in vivo* efficacy of phthalocyanine – nanoparticles conjugates for the photodynamic therapy of amelanotic melanoma"

11h50 - 12h10: Thierry Le Gall (CEA-Saclay)
"Potent antioxidant compounds revealed by a new high-throughput screening method"

12h10 -12h30: Italian Society General Assembly

12h30 - 14h00: Lunch break

14h00 - 15h00: Posters Session

Afternoon symposium: ***Photocarcinogenesis - photoprotection – prevention***

Chairpersons: Leela Daya-Grosjean and Giulio Jori

15h00 - 15h30: Marina Venturini (Brescia)
"Unmet needs for photoprevention of skin cancer"

15h30 - 16h00: Christophe Bedane (CHU Limoges)
"PDT: recent advances in skin disease management"

16h00 - 16h30: Coffee break

16h30 - 16h50: M.T. Rossi (Brescia)
"MAL – PDT off label in real life in Italy"

16h50 - 17h10: Emmanuel Mahé (CHU, Boulogne)
"RISC-UV project, a collaboration between geophysicists and dermatologists"

17h10 - 17h30: Alessia Pacifico (San Gallicano Roma)
"Genetic alteration and skin cancer development after UV radiation"

17h30 - 17h50: Laurent Marrot (L'Oreal)
"Comparison of antioxydant defenses in human epidermal melanocytes and keratinocytes suggests that pigment cells are adapted to photooxidative stress induced by melanin related compounds"

17h50 - 18h10: Giorgia Miolo (Padova)
"Drug-photosensitised modifications of proteins"

20h30: Dinner

Tuesday 26 October

Morning symposium: ***Cellular aspects of biological processes***

Chairpersons: Gianfranco Canti and Laurent Marrot

8h30 - 9h00: Michela Magaraggia (Padova)
"Porphyrin -photosensitised processes: an environmentally friendly approach to water disinfection"

9h00 - 9h30: Jacques Piette (Liège)
"Role of NF-kB in glioblastoma cell death by photodynamic therapy"

9h30 - 10h00: Coffee break

10h00 - 10h20: Marie Dominique Galibert (Univ. Rennes)
"Solar radiation and gene expression: USF in the DNA repair pathway"

10h20 - 10h40: Giuseppe Palumbo (Napoli)
"Mitochondria – proteosome cross talk and photodynamic treatment of lung carcinoma cells"

10h40 - 11h00: Pierre Marie Girard (Institut Curie Orsay)
"Protein oxidation by UVA radiation: the case of PCNA and XRCC3, two DNA binding proteins"

11h00 - 11h20: Justina Sileykite (Padova)
"Opening of the mitochondrial permeability transition pore by porphyrin mediated photooxidative stress is regulated by the interaction of critical external thiols with domains of the translocator protein"

11h20 - 11h40: Jean Luc Coll (IAB, Grenoble)
"Optics and anti-cancer drug delivery"

11h40 - 12h15: Posters presentation

12h15 - 13h45: Lunch break

13h45 - 14h45: Posters Session

Afternoon symposium: ***Environmental photobiology***

Chairpersons: Klaus Brettel and Francesco Lenci

14h45 - 15h15: Massimo Trotta (Bari)
"A different music: photosynthetic bacteria playing heavy metal"

15h15 - 15h45: Ismael Moya (Ecole Polytechnique)
"What remote sensing of vegetation fluorescence can tell us?»

15h45 - 16h15: Coffee break

16h15 - 16h35: Giulio Jori (Padova)
"Effect of porphyrin photosensitization on the constituents of aqueous ecosystems"

16h35 - 16h55: Diana Kirilovsky (CEA Saclay)
"A novel light sensor: a photoactive carotenoid protein"

16h55 - 17h15: Luigi Ceci (CNR Bari)
"Characterization of the spinach Lhcb1 multigene family and its regulatory regions: possible implications in environmental adaptation"

17h15 - 17h35: Stéphanie Bonneau (UMPC, Paris)
"Membrane permeabilization and emergence of Life"

17h35 - 17h55: Francesca Italiano (CNR Bari)
"Interaction of photosynthetic bacteria with chromate: investigation by combining atomic force microscopy, ATR-FTIR spectroscopy and proteomic techniques"

17h55: Closure of meeting

18h05 - 18h35: SFPb General Assembly

ABSTRACTS

- Oral Presentations -

Pyrimidine cyclobutane dimers in DNA: do we know everything after 50 years?

Thierry DOUKI
INAC, SCIB, UJF & CNRS, LCIB (UMR_E 3 CEA-UJF and FRE 3200), Laboratoire « Lésions des Acides Nucléiques », CEA-Grenoble, France

The thymine cyclobutane dimers were first isolated more than half a century ago. Since then, photochemical studies have led to the identification of cytosine-containing cyclobutane pyrimidine dimers (CPDs), as well as that of other dimeric lesions such as pyrimidine (6-4) photoproducts (64PPs). A number of parameters have been identified that may influence the final distribution of dimeric pyrimidine photoproducts. Important secondary reactions, such as deamination and photoconversion of 64PPs into Dewar isomers, were also investigated.

Large amounts of information have also been gathered in cells and skin exposed to UVC and more biologically relevant UVB radiations. Emphasis has been placed on repair of CPDs and 64PPs, and how cell types and different genetic background may modulate this important defence mechanism. Data has also been obtained on the mutagenicity of UVC- and UVB-induced photoproducts. They showed that dimeric pyrimidine photoproducts are highly mutagenic lesions and that CPDs play a more important role than 64PPs.

A large number of points on the formation of pyrimidine dimers remain to be addressed. First, the basic initial steps leading from the excited states to the final damage are still not clearly understood and are under investigation using ultrafast spectroscopic techniques. These experimental approaches are supported by theoretical works.

The recently emphasized formation of CPDs, but not 64PPs, within skin and cells exposed to UVA radiation raises additional questions on the underlying mechanism. This could be a photosensitized process but accumulating evidence for a similar photochemistry in isolated and cellular points toward a direct photochemical process. In agreement, DNA was reported to absorb UVA, although with much lower efficiency than UVB and UVC.

In the field of DNA repair, information is still needed to understand the role of some proteins. In particular, sensing of the damage in chromatin is an important issue. The recent observation that the four different CPDs are not removed from the DNA of irradiated cells with the same rate remains to be explained. A number of questions are also to be answered on the action of specific repair enzymes (photolyases, spore photoproduct lyase) in bacteria and plants.

Altogether, it can be seen that basic photochemical information as well as biological data are still needed to draw a more complete picture of the photobiology of DNA and its link to processes such as skin cancer and impact of UV radiation on ecosystems.

The potential of mel nanoparticles as new nanochromophores for diagnostics, imaging and therapy

Roberto Pini[1], Fulvio Ratto1, Paolo Matteini1, Francesca Rossi1, Sonia Centi2, Franco Fusi2
[1] *Istituto di Fisica Applicata - CNR, Sesto Fiorentino (Italy);*
[2] *Dipartimento di Fisiopatologia Clinica, Università di Firenze (Italy)*

Near IR (NIR) laser-activatable nanoparticles may become a powerful tool in biomedical optics. Because NIR light penetrates deeply into bodily tissues, specific targets stained with these nanoparticles become exposed to efficient and selective laser interaction that may be exploited for imaging, therapeutics, and sensing. Among the alternatives of greatest current interest are gold nanorods. Their excitation involves plasmon resonances (collective-charge oscillations) at NIR frequencies and activates various processes comprising Rayleigh scattering, near-field enhancement, and intense light absorption. As a reference, about 100pM gold nanorods achieve the same extinction in the NIR range as 100μM indocyanine green (ICG), which is among the most efficient NIR dyes. Possible applications for gold nanorods include near-field enhancement for sensing by Raman and luminescence spectroscopy; contrast enhancement for imaging by Rayleigh scattering, fluorescence and photoacoustics; microsurgery by hyperthermia; and photoacoustics. The feasibility of these applications depends on reciprocal interactions between the gold nanorods, NIR light, and the biological environment. In turn, these interactions are affected by the size, shape, and surface modification of the nanoparticles, which govern parameters such as the biodistribution, toxicity, efficiency of photothermal and photoacoustic conversion, stability, and frequency of the plasmon resonances. Colloidal suspensions of gold nanorods may be synthesized by self-assembly and then modified with combinations such as polymers, silicates, and proteins to gain additional functionalities. The scientific community is paying special attention to the conjugation of gold nanorods with antibodies due to its potential for active delivery, such as to malignant cells. As a model example of photothermal therapy, we will present the use of gold nanorods for laser bonding of connective tissues, which is emerging as a powerful alternative to conventional surgical suturing where segments of the skin, blood vessels, organ capsules, and the cornea undergo incision.

References: **1)** Khlebtsov, Dykman, Quant. Spectrosc. Radiat. Transfer 111, 1, 2010; . **2)** Pérez-Juste, Pastoriza-Santos, Liz-Marzán, Mulvaney, Coord. Chem. Rev. 249, 1870, 2005; **3)** Huang, Jain, El-Sayed, El-Sayed, Lasers Med. Sci. 23, 217, 2008; **4)** Tong, Wei, Wei, Cheng, Photochem. Photob. 85, 21, 2009 **5)** Ratto, Matteini, Rossi, Menabuoni, Tiwari, Kulkarni, Pini, Nanomed. 5, 143, 2009 **6)** Takahashi, Niidome, Kawano, Yamada, Niidome, J. Nanopart. Res. 10, 221, 2008 **7)** N. Durr, Larson, Smith, Korgel, Sokolov, Ben-Yakar, Nano Lett. 7, 941, 2007 **8)** Eghtedari, A. Oraevsky, Copland, Kotov, Conjusteau, Motamedi, Nano Lett. 7, 1914, 2007 **9)** Terentyuk, Akchurin, Maksimova, Shantrokha, Tuchin, Maslyakova, Suleymanova, Kogan, Khlebtsov, Khlebtsov, SPIE Newsroom. doi: 10.1117/2.1200907.1619 **10)** Ratto, Matteini, Rossi, Pini, Nanopart. Res., doi:10.1007/s11051-009-9712-0 **11)** Rossi, Matteini, Ratto, Menabuoni, Lenzetti, PiniJ. Biophotonics 1, 331, 2008 **12)** Pini, Rossi, Matteini, Ratto, eds., Biophotonics. Series: Biological and Medical Physics, Biomedical Engineering, p. 275, Springer, Berlin, 2008.

Fluorescence of fullerol in human lens and retinal pigment epithelial cells

Paola Taroni, Cosimo D'Andrea, Gianluca Valentini, Rinaldo Cubeddu
Dipartimento di Fisica, Politecnico di Milano, Milan, Italy

Dan-Ning Hu
Tissue Culture Center, New York Eye and Ear Infirmary, NY

Joan E. Roberts
Department of Natural Sciences, Fordham University, New York, NY

Water-soluble fullerenes [nano-C60(OH)24, fullerol] have recently shown to exhibit antitumor and antiviral activity. They are also being tested as carriers to deliver drugs to the brain, and to by-pass ocular barriers and deliver drugs to specific compartments in the eye.
The fluorescence of fullerol in aqueous environment was preliminary characterized in the spectral and temporal domains.
To explore whether the fluorescence properties of fullerol incorporated into cells correlate with dose (which, in turn, correlates with cytotoxicity1,2), time resolved-fluorescence spectroscopy was performed in vitro on single human lens (HLE) and retinal pigment epithelial (hRPE) cells under a microscope. The intracellular distribution of fullerol and its dependence on concentration was investigated using a system for fluorescence lifetime imaging coupled to the microscope. The concentration of fullerol was varied in the range 1-500 μM for HLE cells and 1-50 μM for RPE cells.
For both cell types the fluorescence decay curves are always best fitted with three lifetimes in the range of 0.15 to 4 ns. The lifetime values change slightly with fullerol concentration, while the amplitude distribution shows more marked dose dependence, in agreement with faster decay curves at high concentrations.
The average emitted fluorescence intensity shows a complex (non-monotonic), but reproducible pattern with fullerol concentration, that seems to correlate with the onset and progression of cell damage due to fullerol cytotoxicity.
Also the spatial distribution of the intracellular fluorescence changes significantly with concentration, following progressive cell damage.

1. Roberts, J.E., Wielgus, A.R., Boyes, W.K., Andley, U., Chignell, C.F., "Phototoxicity and cytotoxicity of fullerol in human lens epithelial cells", Toxicol. Appl. Pharmacol. 228, 49-58, 2008.
2. Wielgus, A.R., Zhao, B., Chignell, C.F., Hu, D.-N., Roberts, J.E., "Phototoxicity and cytotoxicity of fullerol in human retinal pigment epithelial cells", Toxicol. Appl. Pharmacol. 242, 79-90, 2010.

Ultrafast fluorescence spectroscopy studies of electronically excited states in DNA

Thomas Gustavsson
Laboratoire Francis Perrin, CEA/DSM/IRAMIS/SPAM - CNRS URA 2453
CEA/Saclay, 91191 Gif-sur-Yvette, FRANCE

We focus our research on electronically excited states in DNA in order to characterize their structure and dynamics and link these quantities with DNA UV photodamage. To explore the directly populated states, we use fluorescence spectroscopy covering a large time domain, from 100 femtoseconds to nanoseconds.
In this review, various studies ranging from monomeric chromophores to natural DNA through model helices of specific size and sequence will be presented.
While the individual DNA bases are characterized by ultrafast fluorescence lifetimes (<1 ps), the organization of bases into single and double strands leads to much longer lifetimes. This shows that there is a profound change in the nature of the emitting state going from the single base to the double helix.
With the help of high-level quantum chemistry calculations, the ultrafast dynamics of the excited states of single bases has been relatively well understood today. Simply speaking, very efficient non-radiative relaxation channels couple the excited state to the ground state via a conical intersection.
The situation for the helices is more complicated. By comparing experimental data with theoretical predictions, we have demonstrated the importance of the spatial distribution of excited states. In particular, the temporal evolution of fluorescence anisotropy, which occurs on a sub-picosecond timescale, reveals the presence of an ultrafast energy transfer among bases. Fluorescence decays from helices range from the femtosecond to the nanosecond timescale and the relative contribution of the slower components depend strongly on the sequence. For natural DNA, 98 % of the photons are emitted at times longer than 10 ps.

(1) T. Gustavsson, A. Banyasz, E. Lazzarotto, D. Markovitsi, G. Scalmani, M.-J. Frisch, V. Barone, R. Improta, Journal of the American Chemical Society 128 (2006) 607-19.
(2) D. Markovitsi, F. Talbot, T. Gustavsson, D. Onidas, E. Lazzarotto, S. Marguet, Nature 441 (2006) E7.
(3) T. Gustavsson, R. Improta, D. Markovitsi, Journal of Physical Chemistry Letters 1 (2010) 2025-30.
(4) D. Markovitsi, T. Gustavsson, A. Banyasz, Mutation Research/Reviews in Mutation Research 704 (2010) 21-28.
(5) I. Vayá, T. Gustavsson, F.-A. Miannay, T. Douki, D. Markovitsi, Journal of the American Chemical Society (2010).

Quantum Dots nanoparticles for cancer detection

Benoit Dubertret
ESPCI, Paris

The in vivo efficacy of phthalocyanine-nanoparticles conjugate for the photodynamic therapy of amelanotic melanoma

Monica Camerin[1], Michela Magaraggia1, Marina Soncin1, Giulio Jori1, Miguel Moreno2, Isabelle Chambrier2, Michael J. Cook2, David A. Russell2
1 Department of Biology, University of Padova, Padova, Italy
2 School of Chemistry, university of East Anglia, Norwich NR4 7TJ, United Kingdom

The efficiency of a Zn (II)-phthalocyanine disulphide (C11Pc), a phthalocyanine derivative bearing seven hexyl chains and a sulphur terminated C11 chain, as a photodynamic therapy (PDT) agent, was investigated in C57 mice bearing a sub-cutaneously transplanted amelanotic melanoma. These photosensitiser molecules have been shown to exhibit excellent PDT action, although it was necessary to use a cremophor emulsion in order to facilitate their systemic delivery to the animal model. In order to avoid the use of such emulsions we have explored the possibility to use gold nanoparticles as a carrier of the phthalocyanine photosensitiser to tumours.

PDT studies with the C11Pc-loaded amelanotic melanoma showed a markedly more significant response of the tumour in the mice that had received the nanoparticle-bound photosensitiser ; the PDT effect was especially extensive if the irradiation was performed at 3 h after C11Pc injection when large phthalocyanine amounts were still present in the serum. This suggest that the PDT promoted by C11Pc predominantly acts via vascular damage at least in this specific animal model. Ultrastructural studies on PDT-treated tumour specimens confirmed the currency of a predominant vascular damage.

Reference:

The in vivo efficacy of phthalocyanine-nanoparticle conjugates for the photodynamic therapy of amelanotic melanoma, Camerin M, Magaraggia M, Soncin M, Jori G, Moreno M, Chambrier I, Cook MJ, Russell DA.Eur J Cancer. 2010 Jul;46(10):1910-8.

Potent antioxidant compounds revealed by a new high-throughput screening method

Thierry Le Gall
CEA-Saclay, IBiTec-S, Service de Chimie Bioorganique et de Marquage
Bât. 547, 91191 Gif-sur-Yvette cedex, France

A high-throughput screening method for the selection of water-soluble antioxidant compounds will be presented. This method, which uses conventional immunoassay techniques, allows to evaluate the capacity of a given compound to protect the DNA base thymidine against several oxidative conditions, including γ radiolysis.[1] By applying this assay to a library of compounds, the powerful antioxidant activity of the mushroom pigment norbadione A, related to the family of pulvinic acids, was demonstrated. However, a pro-oxidant activity of this compound was revealed in the presence of iron salts, while such effect was not observed with natural pulvinic acids. The synthesis of various new pulvinic acids was then carried out;[2] their antioxidant properties will be reported.

References:

1- Meunier, S. *et al.*, *ChemBioChem* 2004, *5*, 832; Meunier, S. *et al.*, *ChemBioChem* 2005, *6*, 1234.
2- Desage-El Murr, M. *et al.*, *Angew. Chem., Int. Ed.* 2003, *42*, 1289; Heurtaux, B. *et al.*, *J. Org. Chem.* 2005, *70*, 1474; Bourdreux, Y. *et al.*, *Tetrahedron* 2008, *64*, 8930; Nadal, B. *et al.*, *Tetrahedron Lett.* 2009, *50*, 2430; Habrant, D. *et al.*, *J. Med. Chem.* 2009, *52*, 2454.

Unmeet needs for photoprevention of skin cancer

Venturini M, Rossi MT, Sala R, Calzavara- Pinton PG,
Dermatology Department, University of Brescia, Italy

Earth is constantly irradiated by light photons coming from the sun; 56% are infrared light photons (wavelength, 780-5000 nm), 39% visible light (400-780 nm), and 5% UV light (290-400 nm). UV radiation is absorbed by different chromophores in the skin, such as melanin, DNA, RNA, proteins, lipids, water, aromatic amino acids, such as tyrosine and tryptophan; trans-urocanic acid; etc. Absorption of UV photons by these chromophores results in different photochemical reactions and secondary interactions involving reactive oxygen species (ROS), which result in harmful effects.
UVB (290 to 320 nm) is the major wavelength that causes sunburn, and this portion of UV rays directly damages the cellular DNA leading to the formation of the 6-4 cyclobutane pyrimidine dimmers. By contrast, UVA (320 to 400 nm) penetrates deeper into the skin layer and indirectly damages the DNA via the production of radical oxygen species (ROS). Chronic UV exposure has been linked to the development of actinic keratosis, squamous cell cancer, and basal cell cancer, whereas intermittent and intense UV exposure is associated with the development of melanoma, the most dangerous type of skin cancer. UV radiation also plays a major role in the acceleration of the photoaging, clinically, consisting of wrinkling, dryness, telangiectasia, sagging, and pigmentation.
Photoprotection is an essential prophylactic and therapeutic element to decrease photocancerogenesis and photoaging. The development of photoprotection has been stimulated by a change in the behavioral habits of human society. Sunscreen use decreased the number of actinic keratoses and squamous cell cancers, but epidemiologic studies are mixed with some showing preventive benefit, whereas others showing an increased risk for developing basal cell carcinoma and melanoma in patient that routinely used sunscreens.
Many confounding factors may explain the lack of protective effect with routine sunscreen use, such as photoinstability, unmeet among of sunscreen used, no or little UVA protection, etc.
However the same part of the UV spectrum causing skin cancer is required for vitamin D photosynthesis, that is important to bone metabolism and to prevent colon, breast, prostate, and non-Hodgkin lymphoma.
In conclusion it is necessary to emphasize the need of maintaining an appropriate balance between sun avoidance and exposure to minimize skin cancer and promote vitamin D synthesis, respectively.

Photodynamic therapy: Recent advances in skin disease management

Christophe Bédane MD, PhD
Department of Dermatology; Hopital Dupuytren Limoges France

Topical photodynamic therapy (PDT) is now well established for the treatment of various oncologic and non oncologic skin diseases. PDT has demonstrated high efficacy, minimal side effects, and improved cosmetic outcome when used for the treatment of actinic keratoses (AK), basal cell carcinoma and bowen's disease. The development of well tolerated cutaneous photosensitizers like 5-aminolevulinic acid or its methyl ester has enhanced the interest for this treatment modality in dermatology. Red light emitted by incoherent lamps or diodes is now widely available by physiciansPublished clinical studies using PDT in the treatment of AKs yield overall efficacy rates ranging from 50% to 71% with one treatment to as high as 88% to 90% with two or more treatments. For superficial BCC, initial clearance rates were 76% to 97%, and for Bowen's disease, initial clearance rates ranged from 72% to 94% overall. Recent publications of comparative studies have reported equivalent cure rates to standard and reference treatments for superficial lesions. Moreover a therapeutical benefit of PDT has been reported in a wide range of inflammatory dermatosis such as acne vulgaris, granuloma annulare, localized scleroderma or benign familial pemphigus. The use of PDT for photorejuvenation is a relatively new application of this technology, which has shown promise in improving the appearance of fine lines, pigmentary variation and telangiectasias Very low rates of side effects are reported by patients whereas cosmetic outcome is generally considered as very good or excellent. The advantages of photodynamic therapy include the capacity for noninvasive targeted therapy through topical application of aminolevulinic acid and methyl aminolevulinic acid, with outstanding cosmetic results. Although the theory behind the use of chemical photosensitizers and ultraviolet light to treat a wide variety of skin disorders is straightforward, the practical application of this technology is evolving. Additional research into the precise mechanisms of action for specific photosensitizers and optimal light sources will be highly beneficial to the advancement of this technology. Topical photodynamic therapy should become a widely accepted treatment in the near future among various fields of dermatology.

MAL-PDT off label in real life in Italy

Rossi MT, Venturini M, Sala R, Calzavara Pinton PG.
Dermatology Department, University of Brescia, Italy

Photodynamic therapy (PDT) with methyl-aminolevulinate (MAL) is an approved non- invasive treatment option for basal cell carcinoma, actinic keratosis (the biologic precursor of squamous cell carcinoma, SCC), and in situ SCC (Bowen's Disease).

In a recent investigation from our department, MAL- PDT proved also a valuable, effective and well tolerated treatment option with a good cosmetic outcome for well- differentiated microinvasive (Broders scores I and II; Clark levels I and II) SCCs. In contrast, its use for nodular, invasive (Clark level >III) and poorly differentiated (Broders score III and IV) SCC should be discouraged and, if surgery is not applicable, other therapeutic alternatives, e.g. radiotherapy or laser therapy, should be used.

Beside targeting skin tumors of the keratinocyte's lineage, MAL-PDT of sun damaged skin offers other advantages. It can prevent the further development of new SCCs or BCCs (according to the concept of the field cancerization of heavily sun- damaged skin, particularly in immune-suppressed patients) and can also improve the global appearance of photo-aged skin and the severity of selected manifestations, i.e. mottled hyperpigmentation, fine lines, roughness and sallowness.

Other studies have assessed the efficacy of MAL-PDT in the treatment of mycosis fungoides (MF). The conclusion was that MAL- PDT is useful in the treatment of unilesional MF, MF lesions refractory to PUVA therapy and MF lesions localized in "sanctuary" body areas that cannot be exposed to UV light.

In addition, MAL PDT showed some efficacy in the treatment of several inflammatory diseases, e.g. psoriasis, acne vulgaris, hypertrichosis, scleroderma, granuloma annulare, and others as well as in the eradication of skin infections, e.g. warts, HPV-induced condylomata, mycosis and leishmania.

However, at now, only anecdotic reports or small case series are reported in the literature and we need randomized controlled trial to evaluate its actual efficacy.

We report an overview of use of MAL-PDT off label in Italy at the present time.

RISC-UV project, a collaboration between geophysicists and dermatologists

Emmanuel Mahé
CHU, Boulogne, France

Genetic Alterations and skin cancer development after UV radiation

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

Ultraviolet (UV) radiation present in sunlight 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.

References:

1) Fate of UVB induced p53 mutations in SKH-hr1 mouse skin after discontinuation of irradiation: relationship to skin cancer development. Melnikova VO, Pacifico A, Chimenti S, Peris K, Ananthaswamy HN. Oncogene 2005; 24:7055-63
2) p53 and the pathogenesis of skin cancer. Benjamin CL, Ananthaswamy HN. Toxicol Appl Pharmacol 2007; 224: 241-8
3) p53 tumor suppressor gene: a critical molecular target for UV induction and prevention of skin cancer. Benjamin CL, Ullrich SE, Kripke ML, Ananthaswamy HN. Photochem Photobiol 2008; 84: 55-62

Comparison of antioxydant defenses in human epidermal melanocytes and keratinocytes suggests that pigment cells are adapted to photooxidative stress induced by melanin related compounds.

Laurent Marrot, Jean-Philippe Belaïdi, Laurence Denat, Daniel Duche, Christophe Jones, Philippe Perez, Jérémie Sœur and Jean-Roch Meunier.
L'OREAL, Aulnay sous bois, France

Melanin and its chemical intermediates can both generate and scavenge reactive species. Melanocyte have thus to manage a specific situation towards oxidative stress, especially when exposed to sunlight. For instance oxidative stress induced by UV radiation from a solar simulator (SSUV: 300-400 nm or UVA: 320-400 nm) in cultured human melanocytes was stronger when melanogenesis was stimulated. In fact, photoinduced DNA breakage detected using the comet assay and fluorescence of the specific redox probe Dihydro-Rodhamine123 were enhanced upon UV exposure when melanin content increased. By comparing antioxidant status in human melanocytes and keratinocytes from same donors, we could show that (i) reduced glutathione content was higher in keratinocytes, (ii) basal expression of NQO1 (mRNA and protein) was higher in melanocytes, (iii) when Nrf2 was stimulated (by sulforaphane, lipoïc acid or by silencing of Keap1), HO1 (mRNA and protein) and modulatory subunit of γ-glutamyl-cysteine-ligase (GCLm, mRNA) were mainly induced in melanocytes whereas catalytic subunit of GCL (GCLc) was over expressed in keratinocytes, (iii) in a microarray assay, HO1, ferritin, catalase as well as genes from NQO or GST family displayed a stronger basal expression in melanocytes whereas genes from GPX family where mainly expressed in keratinocytes. Melanogenesis could influence such differences through endogenous generation of quinones and hydrogen peroxide. These data are of importance in order to better understand how skin can cope with environmental oxidative stress.

Drug-photosensitised modifications of proteins

G. Miolo and S. Caffieri
Department of Pharmaceutical Sciences, University of Padova, Italy

Several topical or systemic drugs may result in some photosensitivity reactions under light exposure. Wavelengths within the UV-A (320-400 nm) range are more likely to cause drug-induced photosensitisation, although occasionally UV-B (290-320 nm) can also be responsible for such effects. Proteins are one of the main biological targets of this damage. The mechanisms involved in chemical changes of amino acids and proteins upon irradiation are mediated by radicals (Type I), drug-derived peroxides, singlet oxygen (Type II) and direct binding (Type III), giving rise to drug-protein photoadducts with nucleophilic amino acids (e.*g.,* serine, tyrosine, lysine), protein photocross-linking (drug-protein or protein-protein), photodegradation or photooxidation of amino acids (e.g*.,* tryptophan, tyrosine, cysteine/cystine, phenylalanine). The photosensitised modification of proteins and enzymes could lead to loss of their biological functions with damage to some organs and the occurrence of phototoxic side effects as well as to photoallergic reactions when the immune system is involved in the skin. Phenothiazines (fluphenazine, chlorpromazine, promethazine), non selective NSAIDs (tiaprophenic acid) and coxibs (celecoxib), diuretics (hydrochlorothiazide), antitumor agents (5-fluorouracil), corticosteroids (fluocinolone, flumethasone), chlorochresol, are examples of pharmaceutical compounds able to induce protein modifications under UV irradiation.

References:

1. H. Alenius, D. W. Roberts, Y.Tokura, A. Lauerma, G. Patlewicz and M. S. Roberts, Skin, drug and chemical reactions, Drug Discovery Today: Disease Mechanisms, Vol. 5, No.2, e211-e220 (2008)
2. Miranda M.A. J.V. Castell, D. Hernandez et al., Drug-photosensitized protein modification: Identification of the reactive sites and elucidation of reaction mechanisms with tiaprofenic acid/albumin as model system, Chem.Res. Toxicol., 11, 172-177 (1998)
3. Miranda M.A. J.V. Castell, D. Hernandez et al., Mechanisms of Photosensitizion by Drugs: Involvement of tyrosines in the photomodification of proteins mediated by tiaprofenic acid in vitro, *Toxicol. In Vitro*, 11 , 653-659 (1997)
4. Caffieri S., Miolo G., Seraglia S., Dalzoppo D., Toma F.M., van Henegouwen G.M., Photoaddition of fluphenazine to nucleophiles in peptides and proteins. Possible cause of immune system side effects, Chem. Res. Toxicol., 20, 1470-6 (2007)

Porphyrin-photosensitised processes: an environmentally friendly approach to water disinfection

Michela Magaraggia, Clara Fabris, Monica Camerin, Marina Soncin, Giulio Jori, Olimpia Coppellotti, Laura Guidolin.
Department of Biology, University of Padova, Padova, Italy

Saprolegniasis represents an infection of freshwater fish and eggs caused by the water mold *Saprolegnia parasitica* and causes large economic losses in aquaculture. Formalin is the only fungicide registered for use in aquaculture that could replace malachite green, which is currently banned for its carcinogenic potential. However, there are concerns about its effects on the environment, as well as on fish and consumer health. Therefore, the search for alternative fungicides is highly important. This study aims at investigating the potential of porphyrin-type photosensitizers to combat saprolegniasis using (a) cell cultures of bacterial, fungal and parasite pathogen (b) pilot aquaculture plants involving either rainbow trout (*Oncorhynchus mykiss*) and trout eggs. The results obtained by using a cationic porphyrin and low intensity visible light showed an extensive (up to 6-7 log) decrease in the bacterial and fungal population after short incubation and irradiation times in the presence of micromolar photosensitiser concentrations.

Detailed studies were carried out in order to study the possible damages of the treatment to vital organs such as fish gills. In particular fluorescence microscopy and histological analyses were focused on these tissues. Moreover particular attention was assessed on the possible subsequent toxic effects of the treatment on non-target water organisms such as crustacean and protozoan.

Preliminary results indicate that the treatment with cationic porphyrins appeares to be efficient against bacterial and fungal population with no detectable effect on embryo morphology and trout physiology.

The procedure appears to be of low cost and to have a low environmental impact.

Role of NF-kB in glioblastoma cell death by photodynamic therapy

Isabelle Coupienne, Sébastien Bontems & Jacques Piette
Laboratory of Virology & Immunology, University of Liège, B-4000 Liège

NF-kB is a transcription factor which acts as a master controller of the immune system and has also recently shown to be important for the progression and dispersion of solid tumors. Our laboratory has been among the very first one to demonstrate that photodynamic therapy (PDT) can rapidly lead to the nuclear translocation of NF-kB in several cancer cell lines with the subsequent transactivation of genes encoding cytokines and chemokines. The mechanism by which PDT activates NF-kB is still under debate but it seems to depend on the cancer cell type, the nature of the photosensitizer and the cancer cell genetic background. Among the photosensitizers, pheophorbide derivatives are the most efficient because they can monopolize the transduction machinery under the control of the interleukin-1 receptor to rapidly activate NF-kB. ALA-PDT is also capable of efficiently activating NF-kB in various cancer cells but the mechanism remains unclear. Among the physiological consequences associated with the nuclear translocation of NF-kB in cancer cells treated by PDT, resistance to apoptosis is clearly of importance. Indeed, we have generated cancer cell lines where NF-kB activation is impossible and these cells turned out to be more sensitive to cell death. This property is also observed in glioblastoma cells even if these cells are deficient in apoptosis. In this case we demonstrated an important role of the IKK complex which is a central piece in the NF-kB activation process. Inhibition of this kinase complex not only sensitizes glioblastoma to PDT-induced cell death but it also interfere with the onset of autophagy which has been identified as a process that many cancer cells have used to better survive in many conditions. From the data accumulated by our laboratory and others, it is now obvious that NF-kB or IKK inhibition by pharmacological agents should be considered as an important adjuvant for cancer cell eradication by PDT.

Solar radiation and gene expression: USF in the DNA repair pathway

Galibert MD[1], Corre S1, Baron Y1, Mouchet N12, Bouafia A1, Prince S3, Vaulont S4.

[1] *CNRS UMR 6061 Institute of Genetics and Development of Rennes, Tanscriptionnal Regulation and Oncogenesis team, IFR140 GFAS, Faculté de Médecine, Université de Rennes1, FRANCE*
[2] *PROCLAIM Company, Rennes, FRANCE*
[3] *University of Cap Town, SOUTH AFRICA*
[4] *Institut Cochin, Faculté de Médecine Cochin-Port Royal, Paris, FRANCE*

Skin exposure to solar radiation initiates complex molecular processes. These include the protective tanning response, local inflammation, immune suppression, and DNA damage that can lead to skin carcinogenesis. Understanding how transcription factors interpret the output from signal transduction pathway to drive distinct programs of gene expression is a key issue that underpins development and disease. In this context, we could show that the ubiquitously expressed basic-Helix-Loop-Helix Leucine Zipper Upstream Stimulating Factor-1, USF1 is a key player of the UV-response. Following UV irradiation, USF1 transcriptional activity is modulated in a p38-dependent pathway to promote gene expression (Galibert et al., 2001; Corre et al., 2004; Corre et al., 2009). Because USF1 binds specifically E-box regulatory elements (CANNTG) present widely over the genome and do also mediate transcription through protein-protein interaction, USF1 is proposed to regulate a wide number of gene networks (Corre and Galibert., 2005).
To explore further the function of USF1 in mediating the UV-response, we have focus on the identification of new target genes in the DNA-repair pathway, using a combination of in vivo and in vitro assays that were validated with the USF1 knock out mice.

References

- **GALIBERT MD**., CARREIRA, S., GODING, C.R. The Usf-1 transcription factor is a novel target for the stress responsive p38 kinase and mediates UV-induced *Tyrosinase* expression. *EMBO J.* 2001, 20, 5022-5031.
- CORRE S, PRIMOT A, SVIDERSKAYA E, BENNETT DC, VAULONT S, GODING CR, GALIBERT MD. UV-induced expression of key component of the tanning process, the POMC and MC1R genes, is dependent on the p-38 activated upstream stimulating factor-1 (USF-1*). J. Biol. Chem*., 2004, 279(49):51226-51233.
- CORRE S, GALIBERT MD. Upstream stimulating factors: highly versatile stress-responsive transcription factors. *Pigment Cell Res.*, 2005, 18(5):337-348.
- CORRE S, PRIMOT A, BARON Y, LE SEYEC J, GODING C, **GALIBERT MD**. Target gene specificity of USF-1 is directed via p38-mediated phosphorylation dependent acetylation. *J. Biol. Chem*. 2009 Jul 10;284(28):18851-62.

Mitochondria – proteosome cross talk and photodynamic treatment of lung carcinoma cells

Chiaviello A and Palumbo G.

Department of Patologia e Biologia Cellulare e Molecolare, University FEDERICO II, Naples, ITALY.

Photodynamic therapy (PDT) relies on the selective uptake of a photosensitizing molecule in a tumor followed by exposure to suitable light to activate the photosensitizer. The energized photosensitizer interacts with molecular oxygen to produce cytotoxic, short-lived species known oxygen radicals. These species aspecifically react at the site of their formation causing injury to the cell and elicit, from time to time, apoptotic response, necrosis or both. Several lines of *in vitro* evidences have shown that PDT may be conveniently combined with other therapies to improve the overall curative effects. We show that mild photodynamic treatment combined with sub-therapeutic doses of various anti-proteasome drugs results in massive apoptosis in two human lung cancer cell lines, namely NCI-H1299 and A549, which are p53 -/- and p53+/+, respectively. To explain this effect, having observed that photodynamic treatment induced an “transient” stall proteasome activity, we hypothesized that the pharmacologic maintenance of PDT-induced proteasome arrest was responsible of the induction of apoptosis. To prove or rebut this hypothesis we thought that it was important to establish in which intracellular site Photofrin accumulates since this is the place where the light-generated radicals cause the first and greatest injury. In this regard our data, based in part on confocal microscopy, demonstrated that mitochondria are an important intracellular target of Photofrin. Indeed Photofrin accumulates in mitochondria and, in accord with this fact, we could demonstrate that a sub-lethal irradiation caused a rapid and significant decrease of mitochondrial membrane potential ($\Delta\Psi m$). This effect was not irreversible since 6 hours post PDT the mitochondrial membrane potential recovered in full. According to these observations, our hypothesis that Photofrin–PDT directly affected the proteasome machinery has been revised. We propose that the oxidative stress induced by PDT in mild conditions induces a transient dysfunction of mitochondria and this in turn leads to malfunctioning of the proteasome. This conclusion has been further strengthened by several experiments with specific inhibitors of respiratory chain complexes (namely I-III). The role of mitochondria is to produce ATP through respiration; this ATP is used for all metabolic activities including those needed for the degradation of proteins by proteasome. Then is the PDT-mediated mitochondrial dysfunction that induces the proteasome block while a prolonged arrest, whatever generated, induces apoptosis.

Protein oxidation by UVA radiation: the case of PCNA and XRCC3, two DNA binding proteins

Pierre-Marie Girard[a,b], Dany Graindorgea,b, Evelyne Sagea,b.
[a] *CNRS UMR3348, Centre Universitaire, 91405 Orsay, France*
[b] *Institut Curie, Bât. 110, Centre Universitaire, 91405 Orsay, France*

Ultraviolet A (320-400 nm) radiation constitutes more than 90% of the UV radiation reaching Earth's surface. It contributes to photodermatosis, skin aging, and likely to skin carcinogenesis (4). Exposure to UVA induces the formation of photoexcited states of cellular photosensitizers with subsequent generation of reactive oxygen species (ROS), mainly constituted of singlet oxygen, leading to damages to membrane lipids, proteins and nucleic acids (6). Although over the last 15 years, we and others have particulary focused on UVA-induced DNA damage (2), recent published data (1, 3, 5) prompted us to explore the possibility that protein oxidation contributes to a certain extent to the overall harmful effects of UVA radiation.

To highlight this point, we carried investigations on two proteins involved in DNA metabolism, Proliferating Cell Nuclear Antigen (PCNA), an homotrimeric cofactor of DNA polymerases, and XRCC3 involved in homologous recombination. We found that UVA-induced ROS i) generate an irreversible covalent bound between the three subunits of PCNA, leading to a stable PCNA trimer, as previously reported (1, 5) and ii) cause reversible disulfide bound formation in XRCC3. While the consequences of irreversible (PCNA) or reversible (XRCC3) oxidation on the functionality of theses proteins have not yet been fully investigated, these data emphasize that protein oxidation may play a role in genome instability.

1. **Bae SI, Zhao R, and Snapka RM.** PCNA damage caused by antineoplastic drugs. *Biochem Pharmacol* 76: 1653-1668, 2008.
2. **Cadet J, Sage E, and Douki T.** Ultraviolet radiation-mediated damage to cellular DNA. *Mutat Res* 571: 3-17, 2005.
3. **Girard PM, Pozzebon M, Delacote F, Douki T, Smirnova V, and Sage E.** Inhibition of S-phase progression triggered by UVA-induced ROS does not require a functional DNA damage checkpoint response in mammalian cells. *DNA Repair (Amst)* 7: 1500-1516, 2008.
4. **Krutmann J.** Ultraviolet A radiation-induced biological effects in human skin: relevance for photoaging and photodermatosis. *J Dermatol Sci* 23 Suppl 1: S22-26, 2000.
5. **Montaner B, O'Donovan P, Reelfs O, Perrett CM, Zhang X, Xu YZ, Ren X, Macpherson P, Frith D, and Karran P.** Reactive oxygen-mediated damage to a human DNA replication and repair protein. *EMBO Rep* 8: 1074-1079, 2007.
6. **Wondrak GT, Jacobson MK, and Jacobson EL.** Endogenous UVA-photosensitizers: mediators of skin photodamage and novel targets for skin photoprotection. *Photochem Photobiol Sci* 5: 215-237, 2006.

Opening of the mitochondrial permeability transition pore by porphyrin-mediated photooxidative stress is regulated by the interaction of critical external thiols with domains of the translocator protein

Justina Šileikytė[1], Valeria Petronilli1, Peter Nikolov2, Paolo Bernardi1 and Fernanda Ricchelli3
[1]*C.N.R. Institute of Neurosciences at the Department of Biomedical Sciences, University of Padova, Italy;* [2]*Bulgarian Academy of Sciences, Institute of Organic Chemistry, Sofia, Bulgaria;* [3]*C.N.R. Institute of Biomedical Technologies at the Department of Biology, University of Padova, Italy*

Hematoporphyrin (HP)-mediated photooxidative stress can either prevent or activate the mitochondrial permeability transition (PT) depending on the site of porphyrin/target localization. HP situated in matrix-exposed sites of the PT pore (PTP) promotes photosensitization of key histidines (His), thereby leading to PT inhibition. PT re-activation can be achieved by photomodification of external cysteines (Cys). Studies with isolated inner membrane (mitoplasts) prepared by digitonin treatment indicated that PT-activating Cys are located on the outer side of the inner membrane. In analogy with the results obtained in intact mitochondria, irradiation of HP-treated mitoplasts at low light doses caused PT inhibition that was counteracted by diethyl pyrocarbonate, indicating that it resulted from photomodification of PTP-regulating His residues. Unlike mitochondria, however, irradiated HP-loaded mitoplasts did not undergo PT re-activation via external Cys modification after the block caused by His photodegradation. This would suggest that, in mitochondria, thiol-sensitizing HP interfered with PTP domains from a site of the outer membrane. HP is known to interact with mitochondria specifically via the outer membrane-bound translocator protein-18 kDa (TSPO, formerly known as the peripheral-type benzodiazepine receptor), as it is typical of dicarboxylic porphyrins endowed with protoporphyrin IX (PP) configuration. Other PP-like dicarboxylic porphyrins, such as deuteroporphyrin (DP) and PP itself, promoted photooxidative effects on the PTP comparable to those of HP, whereas PP-unrelated porphyrins were ineffective. In addition, in the presence of PP-like dicarboxylic porphyrins the PT occurring through modification of external Cys was antagonized by N,N-dihexyl-2-(4-fluorophenyl) indole-3-acetamide (FGIN1-27), a porphyrin-competitive, specific TSPO-ligands, indicating that the binding domains of external thiols do interfere with TSPO regions linking the porphyrin and FGIN1-27 at vicinal sites. Taken together, these findings suggest the involvement of specific TSPO regions in the regulation of the mitochondrial PT.

Optics and anti-cancer drug delivery

Dufort S[1]., Sancey L[1]., Josserand V[1]., Keramidas M[1]., Righini C[1]., Dinten JM[2], Texier-Nogues N.[2], Hirsjarvi S[3], Passirani C[3], Benoit JP[3], Roux S[4], Tillement O[4], Boturyn D[5], Dumy P[5], Coll JL.[1]

1 :INSERM U823, Grenoble; 2 : CEA-LETI Grenoble; 3 : INSERM U646, Angers; 4 : CNRS UMR5260, Lyon and UMR6213 Besançon; 5 : CNRS UMR 5250 Grenoble

Early and accurate detection of tumors, like the development of targeted treatments, is a major field of research in oncology. The generation of specific vectors, capable of transporting a drug or a contrast agent to the primary tumor site as well as to the remote (micro-) metastasis would be an asset for early diagnosis and cancer therapy. Our goal is to develop new treatments based on the use of tumor-targeted delivery of large biomolecules (DNA, siRNA, peptides). We generated new targeting vectors (as the RAFT-RGD) and nanoparticles for drug and biomolecules delivery. To undertake this work, we also developed several optical imaging systems allowing the follow-up and evaluation of our vectorisation systems.

Near-infrared fluorescence (NIR ; 650-900 nm) can be imaged in 2D or 3D. The strong reflection of incident light and autofluorescence of the skin affect the sensitivity when working in reflectance. Switching to Fluorescence Molecular Tomography (FMT) mode greatly improves the quality of whole-body fluorescence imaging. It offers 3D volumetric imaging, true quantification very little affected by depth, optical tissue properties and heterogeneity, and autofluorescence. It is thus an emergent diagnostic tool for the localization and quantification of fluorescent probes, at a depth of a few cms, in some organs, like breast or prostate. In such situations, this technique may be considered as an alternative to the classical ionizing radiation imaging techniques, or a complement to morphological imaging as ultrasounds. Very recently, clinical applications in surgery of cancer as well as for the diagnosis of human prostate cancer became realistic. All these recent issues will be presented.

Reférences 2010

1. Keramidas, Josserand, Righini, Wenk., Faure and Coll: Intraoperative near-infrared image-guided surgery of peritoneal carcinomatosis in a preclinical mouse model. *British Journal of Surgery*, 2010 May;97:737-43
2. Rou, Tillement, Billotey, Coll, Le Duc, Marquette, Perriat: Multifunctional nanoparticles: from the detection of biomolecules to the therapy. *Int J Nanotechnol.* In Press
3. Jin, Furukawa, Waki, Akaji, Coll, Saga and Fujibayashi: Effect of Multimerization of a Linear RGD Peptide on Integrin Binding Affinity and Specificity. *Biol. Pharm. Bull.* . 2010;33:370-8.
4. Koenig, Hervé, Josserand, Berger, Boutet, Da Silva, Peltié, Coll, and Dinten. Fluorescence diffuse optical tomograph for "free space" and multi-fluorophore studies. *J. Biomed. Optics*. Vol. 15, 016016 (2010)
5. Dufort, Sancey, Wenk, Coll. Optical small animal imaging in the drug discovery process. *Biochim. Biophys. Acta-Biomembranes,* 2010 Mar 23. in press
6. Goutayer, Dufort, Josserand, Royere, Heinrich, Vinet, Bibette, Coll, Texier: Tumor targeting of functionalized lipid nanoparticles : Assessment by *in vivo* fluorescence imaging. *Eur. J. Pharmac. Biopharmac.,* 2010 in press
7. Galibert, Sancey, Renaudet, Coll, Dumy, Boturyn. Application of click-click chemistry to the synthesis of new multivalent RGD conjugates. *Org Biomol Chem.* 2010 Sep 9. in press
8. Valero, Sancey, Kucharczak, Guillemin, Gimenez, Prudent, Gillet, Salgado, Coll, Aouacheria. Bax-derived membrane active peptides act as potent and direct inducers of apoptosis in cancer cells. *J. Cell Science*, In press

A different music: photosynthetic bacteria playing heavy metal

Massimo Trotta

Istituto per i Processi Chimico Fisici – Consiglio Nazionale delle Ricerche. Bari

Heavy metal pollution is a very sensitive environmental topic that has an enormous impact on public opinion. Several traditional chemical and physical techniques are employed in tackling this problem, often originating from industrial or mining activities. Recently, next to such techniques, the possibility of using the microbial word in heavy metal polluted sites has been proposed, and scientific literature is now witnessing an increase of publication in this field. The on-line resource of the National Institute of Health (http://www.ncbi.nlm.nih.gov/pubmed) founds over 840 hits upon searching for *bioremediation* in the article title starting from 1988 when such definition appears for the first time. A very recent review by Gadd [1], one of the pioneers of *bioremediation* of heavy metals, gives an up-to-date description of the state-of-the-art of the field, including example of photosynthetic organisms, plants and algae. Of course the grass is not always greener on the other side of the river, but this approach has shown promising results that have even spurred entrepreneurial activity in USA and northern Europe.

In this broad framework, anoxygenic photosynthetic bacteria are being tested since 2005 in our laboratory [2-7] as tool in heavy metal removal from polluted sites, and the effects of several metal ions and oxyanions on the metabolism of *R. sphaeroides* have been scrutinised.

The state-of-the-art of our research on several heavy metals will be presented along with future directions. Furthermore the European framework in which the research is presently developing will also be presented.

References

1. Gadd, G.M., *Metals, minerals and microbes: geomicrobiology and bioremediation.* Microbiology. **156**(3): p. 609-643.
2. Buccolieri, A., et al., *Testing the photosynthetic bacterium Rhodobacter sphaeroides as heavy metal removal tool.* Ann Chim -Rome, 2006. **96**: p. 195-204.
3. Giotta, L., et al., *Heavy metal ion influence on the photosynthetic growth of Rhodobacter sphaeroides.* Chemosphere, 2006. **62**(9): p. 1490-9.
4. Giotta, L., et al., *Magnesium chemical rescue to cobalt-poisoned cells from Rhodobacter sphaeroides*. 2008, Dordrecht: Springer.
5. Italiano, F., et al., *Response of the carotenoidless mutant Rhodobacter sphaeroides growing cells to cobalt and nickel exposure.* International Biodeterioration & Biodegradation, 2009. **63**: p. 948–957.
6. Italiano, F., et al., *Effect of cobalt ions on the soluble proteome of a Rhodobacter sphaeroides carotenoidless mutant*, in *Energy from the sun. 14th International Congress on Photosynthesis.*, G. Allen J., E. Goldbeck J., Osmond B. , Editor. 2008, Springer: Dordrecht. p. 1479-1484.
7. Pisani, F., et al., *Soluble proteome investigation of cobalt effect on the carotenoidless mutant of Rhodobacter sphaeroides.* Journal of Applied Microbiology, 2009. **106**(1): p. 338-49.

What remote sensing of vegetation fluorescence can tell us?

Ismaël Moya, Y. Goulas, A. Ounis, F. Daumard, A. Fournier and S. Champagne
Laboratoire de Météorologie Dynamique (L.M.D), Ecole Polytechnique, 91128 Palaiseau

Terrestrial vegetation is strongly coupled to atmospheric processes through energy, water and carbon exchange. It is apparent that even minor changes in ecosystem scale photosynthesis can have a major effect on the global carbon balance, so that improved knowledge of vegetation photosynthesis at the ecosystem scale clearly becomes a priority in research about the Earth System.
Until now, most of the information that has been acquired by remote sensing of the Earth's surface about vegetation conditions has come from "reflected" light in the solar domain. There is, however, one additional source of information about vegetation in the near-infrared wavelength range that has not yet been exploited. It is related to the emission of fluorescence from the chlorophyll of assimilating leaves: part of the energy absorbed by chlorophyll is not used for photosynthesis, but re-emitted as fluorescence.
Solar-induced fluorescence can be measured by passive techniques, making use of strong atmospheric absorption bands in narrow regions of the spectrum, where apparent vegetation reflectance is significantly contributed by chlorophyll fluorescence. Recent studies have demonstrated that the weak fluorescence signal is detectable from an airborne platform and possibly from a satellite system.
However, the contribution of several perturbing effects should be evaluated and modelled before retrieving a fluorescence signal related to the photosynthetic activity. These include atmospheric corrections, 3D vegetation structure, leaf anatomy and chlorophyll content.

Effect of porphyrin photosensitisation on the constituents of aqueous ecosystems

Giulio Jori, Michela Magaraggia, Clara Fabris, Monica Camerin, Marina Soncin, Olimpia Coppellotti, Laura Guidolin.
Department of Biology, University of Padova, Padova, Italy

Porphyrin-photosensitised processes are increasingly used for an environmentally friendly approach to water disinfection owing to (a) their efficient action against a variety of microbial pathogens which are responsible for the development of water-borne diseases; and (b) the selectivity of these photoprocesses which allows their use in fish-farming plants without affecting fish. However, some concern raises because of the possible photodamage to non-target constituents of aqueous ecosystems.
We undertook a systematic investigation to assess the level of photosensitivity exhibited by selected representatives of those organisms which are commonly present in aqueous media with particular attention to freshwater environments. A tetracationic porphyrin, namely meso-tri(N-methyl-pyridyl), mono(N-dodecyl-pyridyl) porphine (C12), was used as the photosensitiser; this porphyrin has been proposed as a water photodisinfectant in aquaculture and is being explored as a larvicidal agent. The effect of this porphyrin both in the dark and upon exposure to visible light has been tested against two typical parasitic protozoa, such as *Colpoda inflata* and *Tetrahymena thermophila*. Both trophozoites and cysts of *C. inflata* were exposed in the dark for 1 h to 0.1–10 μM porphyrin concentrations and subsequently irradiated with 400-800 nm light (10 mW/cm2). The cysts were fairly resistant to the porphyrin in the dark: only upon incubation with porphyrin doses as large as 6 μM a significant inhibition of excystment was observed (LD50 about 3 μM). A strong phototoxic effect was induced by the combined action of the porphyrin and visible light so that the LD50 decreased to 0.3 μM. As regards the trophozoites, little mortality was caused by the dark incubation with 0.3–10 μM C12. Only in the presence of porphyrin doses > 3 μM some abnormal behaviour (e.g., decreased motility, elongated cellular shape) was observed. The exposure of the trophozoites to visible light was accompanied by a marked phototoxic effect, starting with porphyrin doses of 0.6 μM. No residual survival occurred for 1 μM porphyrin. These observations emphasize the opportunity to build a detailed data-base on the photosensivity of a broad number of such organisms before porphyrin-photosensitised processes can be safely applied to address environmental problems.

A novel light sensor: a photoactive carotenoid protein

A Wilson, C Boulay, M Gwizdala, C Punginelli and D Kirilovsky

Commissariat à l'Energie Atomique (CEA), Institut de Biologie et Technologies de Saclay (iBiTec-S) and Centre National de la Recherche Scientifique (CNRS), 91191 Gif sur Yvette, France.

The water-soluble orange carotenoid protein (OCP), a cyanobacterial protein of 35kDa which binds a single keto-carotenoid was first described and isolated by Holt and Krogman in 1981. During long time its role was unknown. In 2006 we demonstrated that OCP is essential in a photoprotective mechanism in cyanobacteria. Cyanobacteria protect themselves from intense sunlight by dissipating excess absorbed energy as heat at the level of their extramembranal antenna, the phycobilisomes. This mechanism involves a specific decrease of the fluorescence emission of the phycobilisomes and a decrease of the energy transfer from the phycobilisomes to the reaction centers. The process is induced by the absorbance of blue-green light by the OCP which acts as the light intensity sensor and is a new member of the family of photoactive proteins. It is the first photoactive protein containing a carotenoid as the photoactive chromophore and its photocycle is completely different from those of other photoactive proteins. The absorbance of blue-green light by the OCP induces structural changes in the carotenoid and the protein, converting its dark stable orange form (OCPo) into a relatively unstable active red form (OCPr). OCPr form is essential for the induction of the photoprotective mechanism. In the absence of the formation of the red form due to point mutations in the OCP or to the binding of zeaxanthin, no fluorescence quenching is induced by strong light. The tyrosines and tryptophans interacting with the carotenoid rings are essential for photoactivity. The red form is essential but not sufficient. Specific conformational changes occurring in the protein seem to be critical to the events leading to energy dissipation. In darkness, OCP^r spontaneously reverts into the orange form and, *in vivo,* a recovery of the lost fluorescence and of the full capacity of the antenna is observed. However, the fluorescence recovery kinetics *in vivo* are slower than the OCP^r to OCP^o dark conversion *in vitro*, suggesting that the OCP^r form is more stable *in vivo* than *in vitro*. This OCP^r stabilization could be explained by a specific interaction of OCP^r with the phycobilisomes. Our previous results suggested that an unknown protein might be involved in the detachment of the OCP^r from the phycobilisome and in the destabilization of OCP^r . We have just described a novel protein which plays this role being essential for fluorescence recovery after high irradiance exposure. In the absence of this protein, the Fluorescence Recovey Protein (FRP), once illuminated by high irradiance, cyanobacteria cells remain in a low antenna capacity state even after being transferred to low light conditions. Thus, OCP is essential to decrease the energy arriving to the reaction centers under high irradiance and FRP is essential to recover the full antenna capacity once the light intensity decreases.

References 1) Wilson, Ajlani, Verbavatz, Vass, Kerfeld and Kirilovsky (2006) Plant Cell 18, 992 2) Wilson, Punginelli, Gall, Bonetti, Alexandre, Routaboul, Kerfeld, van Grondelle, Robert, Kennis, Kirilovsky (2008) Proc. Natl. Acad. Sci. USA *105*, 12075 3) Wilson, Kinney, Zwart, Punginelli, D'Haene, Perreau, Klein, Kirilovsky, Kerfeld (2010) J. Biol. Chem. 285, 18364 4) Boulay, Wilson, D'Haene, Kirilovsky (2010) Proc. Natl. Acad. Sci. USA 107, 11620

Characterization of the spinach Lhcb1 multigene family and its regulatory regions: possible involvement in environmental adaptation

Claudia Leoni1, Mariateresa Volpicella1, Raffaele Gallerani1,2 and Luigi R. Ceci[2]

[1]*Department of Biochemistry and Molecular Biology, University of Bari, Via Amendola 165/A, 70126 Bari.*

[2]*Institute of Biomembranes and Bioenergetics, CNR, Via Amendola 165/A, 70126 Bari, Italy.*

Photosynthesis in higher plants is carried out by two electrically connected photosynthetic units, PSI and PSII, containing pigment-protein complexes that act as antennae to harvest solar light energy. The light harvesting complex II (LHCII), associated with PSII, contains three highly homologous chlorophyll-*a**b*-binding proteins (Lhcb1, Lhcb2 and Lhcb3), which can be assembled in both homotrimers and heterotrimers. Generally Lhcb1 and Lhcb2 are encoded by multigene families, whose members are variable in number from plant to plant, and whose different function is yet to be clarified.

By RACE analysis, we identified in spinach leaves the full-length cDNAs corresponding to three isoforms of Lhcb1 polypeptides, whose isoforms appear to be differentially expressed in response to long-term white light exposure (1). The three encoded Lhcb1 mature polypeptides are highly homologous to each other, sharing very high identities (97-98%). Only the Lhcb1.1 polypeptide shows an amino acid substitution having a clear functional meaning. It is characterized by a Thr3>Ser substitution which was found to affect phosphorylation level of Lhcb1 polypeptides, a crucial step in the State Transition process (2).

In order to sequence the regulatory regions of the spinach *Lhcb1* genes, we have developed a suitable Genome Walking method which allows the contemporary analysis of members of multigene families (3). By this approach we identified two additional *Lhcb1* genes, not previously hypothesised on the basis of cDNA and proteomic analysis. Gel shift assays of identified regulatory motifs showed the different activity that distinct regulatory motifs may have in the expression of members of the gene family (4). These results, together with the phylogenetic analysis of the *Lhcb1* families, sustain the hypothesis of a role of members of the multigene families and their regulatory regions in the adaptive response of plants to different light conditions.

References:

(1) Rea et. al., 2007 Physiologia Plantarum 130: 167-176.

(2) Allen and Forsberg, 2001 Trend Plant Sci 6: 317-326.

(3) Leoni et. al., 2008 BioTechniques 44: 229-235.

(4) Leoni et. al., 2010 Plant Physiology 167: 138-143.

Membrane permeabilization and emergence of life

Anne-Sophie Herrier, Clémentine Delan-Forino, Christine Vever-Bizet, Claire Torchet and Stéphanie Bonneau

Laboratoire ANBiopHy, CNRS FRE3207, Université Pierre et Marie Curie, Paris

The compartmentalization is one of the essential elements of the current living organisms. Primitive cells - without complex biomachinery present in modern cells - would have had to be based on the self-organizing properties of their components modulated by the interactions with their environment to achieve basic functions such as growth and division. Many vesicles, made of amphiphilic compounds, are able to exhibit complex morphological changes such as growth, fusion, fission, budding. Because of these rich dynamic properties, such vesicles are interesting theoretical models of how primitive cellular life might have occurred and replicate in response to purely physical and chemical forces.

Our study focuses on the structures of compartmentalization (vesicular structures) and the parameters governing their stability. Particular attention is paid to the dynamics of these systems and the effect of light on these dynamics. Within this framework we have shown that, under certain circumstances, light can induce an asymmetry between the leaflets of the membrane and, subsequently, its photo-controlled permeabilization. Using Giant unilamellar vesicles and a chlorin, we asymmetrically photo-damage the membranes. We observed different shape transitions, such as budding, typical of membrane curvature modifications. The asymmetry of the shape transitions are in accordance to a lowered effective spontaneous curvature of the leaflet targeted. This effect is interpreted as a decreased preferred area of the targeted leaflet compared to the other. Permeabilization of the vesicle is interpreted as the opening of a pore above a critical membrane tension. Additionally, asymmetric photo-damaging was shown to be fusogenic.

Our efforts to understand how a minimal cell can function has been continued by establishing conditions that allow Mg2+ dependant-ribozymes to be operative within vesicles. A hammerhead ribozyme was incorporated into vesicles produced in the previously explained manner. The photo-controlled membrane permeabilisation governs the internal level of Mg2+ (initially present in the external compartment) and lead to the activation and self-cleavage of the ribozyme molecules.

Such a combination of stability and dynamics is critical for understanding compartmentalization in a theoretical point of view, and for apprehending models of protocells in the laboratory. Such processes may have been important for early stages of life emergence.

Julien Heuvingh, Stéphanie Bonneau. Biophysical Journal, december 2009, 97(11):2904-2912..

Interaction of photosynthetic bacteria with chromate: investigation by combining atomic force microscopy, ATR-FTIR spectroscopy and proteomic techniques

F. Italiano[1], F. Milano1, A. Agostiano2, L.R. Ceci3 , F. De Leo3, R. Gallerani4, S. Rinalducci5, L. Zolla5, M. Trotta1

[1]Istituto per i Processi Chimico-Fisici (CNR-Bari) , [2]Dipartimento di Chimica – Università degli studi di Bari, [3]Istituto di Biomembrane e Bioenergetica (CNR-Bari , [4] Dipartimento di Biochimica e Biologia Molecolare – Università degli studi di Bari, [5]Dipartimento di Scienze Ambientali, Università della Tuscia, Viterbo

Chromate is a highly soluble and toxic non-essential oxyanion for most organisms. A number of chromate resistant bacteria have been investigated and diverse resistance mechanisms were found [1].

We are investigating the potentialities of the photosynthetic facultative bacterium *Rhodobacter* sphaeroides, known for its ability to tolerate high concentrations of several heavy metal ions [2] and bioaccumulate some of them, such as nickel and cobalt [3, 4], in the bioremediation of chromate polluted sites. Employing an interdisciplinary approach, the response to chromate stress was investigated by combining biochemical and spectroscopic measurements, proteomic characterization and cell imaging.

An efficient resistance mechanism to chromate is suggested both by the high EC50 value and the lag-phase lengthening induced at concentrations above 0.05 mM. *R. sphaeroides* is also able to reduce chromate to the less toxic and soluble form Cr(III) with reductase activity preferentially associated with the protein soluble fraction. Chromate effect on soluble enzymes was investigated by a proteomic approach: soluble protein expression profiles of cells exposed to chromate were compared with those of untreated control cells through two-dimensional gel electrophoresis analysis. Upon exposure to chromate at least 30 soluble proteins were differentially expressed. The wide variety of differentially expressed proteins suggests that different metabolic pathways are involved as response to chromate exposure. The accompanying physiological response to Cr(VI) exposure included marked changes in cellular morphology as revealed by atomic force microscopy.

1. Cervantes, C., et al., *Interactions of chromium with microorganisms and plants.* FEMS Microbiol Rev, 2001. **25**(3): p. 335-47.
2. Giotta, L., et al., *Heavy metal ion influence on the photosynthetic growth of Rhodobacter sphaeroides.* Chemosphere, 2006. **62**(9): p. 1490-9.
3. Italiano, F., et al., *Response of the carotenoidless mutant Rhodobacter sphaeroides growing cells to cobalt and nickel exposure.* International Biodeterioration & Biodegradation, 2009. **63**: p. 948–957.
4. Pisani, F., et al., *Soluble proteome investigation of cobalt effect on the carotenoidless mutant of Rhodobacter sphaeroides.* Journal of Applied Microbiology, 2009. **106**(1): p. 338-49.

ABSTRACTS

- Posters -

Drug photosensitization induced in proteins and in cells

G. Bracchitta[1], A. Catalfo[1], E. Sage[2], P. M. Girard[2], G. De Guidi[1]
[1] Dipartimento di Scienze Chimiche, Università di Catania, 95125 Catania, Italy
[2] Institut Curie, Centre de Recherche, Orsay, F-91400 France

A number of biomolecules is known to undergo extensive changes upon irradiation in the presence of photosensitizers. Certain drugs can behave as photosensitizers producing protein modifications, which are thought to be responsible for the occurrence of photoallergy and other undesired light-induced side effects[1]. In the case of proteins, their photosensitized modifications can lead to loss of biological function (inactivation of enzymes, hormones, etc.). The aromatic amino acid tryptophan is the most susceptible protein residue involved in various photosensitized adverse effects[2]. Between them, the tryptophan photosensitization induced by methylene blue has been well-studied[3]. A predominant type II photosensitizing activity, mediated by singlet oxygen, was previously demonstrated on various models[3]. The purpose of this study is to compare this well-known photosensitization system with that induced by naproxen, a drug belonging to the class of non-steroidal anti-inflammatory drugs. The tryptophan photoproducts represent the biomarkers of the oxidative damage indicative for the molecular mechanism of protein photooxidation[2]. Particular emphasis is dedicated to amino acid modifications caused by the formation of drug photomediated toxic species, such as reactive oxygen species. In the present work, the photochemistry and photosensitization of Trp induced by naproxen and methylene blue on the isolated protein using as model purified bovine serum albumin (BSA) was studied. The results are in agreement with what achieved on the free amino acid[4] and the photosensitization mechanism proceeds via type I and II pathways.
Furthermore, the cellular response in mammalian cells to naproxen itself or in association with UVA radiation were studied. Some preliminary results will be presented.

References

1. Miranda, M. A. and coll. (1998) Chem. Res. Toxicol. 11, 172

2. Wondrak, G. T. and coll. (2006) Photochem. Photobiol. Sci.,5, 215

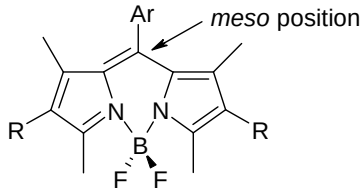
3. Zhi-Ren, L. and coll. (1996) RNA 2 (6), 611.

4. De Guidi, G. and coll. (2009) Photochem. Photobiol. Sci., 1467

Synthesis and photodynamic activity of a series of bodipy a new class of photosensitizers

Stefano Banfi, Enrico Caruso, Marzia B. Gariboldi, Elena Monti, Stefano Zaza
DBSF, Università dell'Insubria, Via Dunant 3, 21100 – Varese (Italy)
email: enrico.caruso@uninsubria.it

Photodynamic therapy (PDT) is now a well-validated modality for the treatment of a number of cancer types. It is based on the systemic or topical administration of a non toxic compound, called photosensitizer (PS), followed by an incubation period to allow PS uptake by the tumor, which is then irradiated with low-energy, tissue penetrating light of the appropriate length to activated the PS. Upon activation, the PS switches from its ground state to an excited singlet state, initiating a chain of electron transitions that results in the production of death-inducing reactive oxygen species (ROS), mainly singlet oxygen ($^1O_2$).
A small number of PSs have been approved for clinical PDT applications on different cancer types, but many more are undergoing clinical trials. For the most part, these new entries retain the cyclic tetrapyrrole structure typical of porphyrin derivatives.
Recently, one alternative class of non-porphyrin PSs has emerged, based on the boron dipyrromethene (BODIPY) fluorophore, exhibiting a number of features that might be exploited for PDT, as well as photodiagnostic, applications in oncology. BODIPY dyes have a wide range of applications, including as fluorescent dyes, sensitizers for solar cells and non linear optical materials. However their possible use as PSs in PDT has only recently been investigated.
BODIPY derivatives are easily synthesized in "one pot" procedure; they have high molar extinction coefficients and high quantum efficiencies of fluorescence, and their activation is relatively unaffected by the variable characteristics (pH, polarity) of different intracellular environments.
In this work we synthesized a series of a new BODIPY with different substituent on the pyrrolic ring and on the *meso* position. The activity of the new PSs was determined *in vitro* on two different tumor cell lines (HCT116 and RT112) through MTT test. For these preliminary studies white alogen lamp (500 W) and green LED were used as light source.



Effect of selenium on lethal and genotoxic UV irradiation and its role on DNA repair system

V. De Rosa[1], A. M. Diamond[2], A. Favier[1], T. Douki[1] and W. Rachidi[1]
[1]*Laboratoire des Lésions des Acides Nucléiques INAC/SCIB-UMR-E 3 CEA-UJF – CEA Grenoble 17 rue des Martyrs – 38054 Grenoble cedex 9 – France*
[2]*University of Illinois at Chicago, Dept. of Pathology, 840 South Wood, Chicago, IL 60612*

Experimental as well as epidemiological data indicate that a variety of nutritional factors can act as anticarcinogens. Their action of some of them is explained by their ability to protect the genome. In particular, exposure to a number of environmental and occupational genotoxic substances such as X-rays, UV light and a variety of chemicals results in an enhanced generation of free oxygen radicals that produce damage to DNA. Selenium (Se) lies among the best known essential elements exhibiting beneficial effects, when used as dietary supplements, on cardiovascular diseases, cancer, thyroid disorders, infertility and in augmenting immune cell performance. Data reported from the Nutritional Prevention of Cancer clinical trial indicated that dietary supplementation with 200 µg/day of Se resulted in a 63% reduction in cancer risk. However SELECT study, stopped in October 2008, has demonstrated that Se and Vitamin E supplementation did not prevent prostate cancer in the generally healthy population. Selenium acts as a cofactor for several antioxidant enzymes such as glutathione peroxidase and thioredoxin reductase. Both of them have been implicated in the protection from ultraviolet (UV) radiation-induced damage. However, mechanisms for selenium-anticancer action are not fully understood; selenium could oppose to toxic effects of free radicals which could be responsible for the genesis of certain malignant diseases.

Because induction of damage to the genome is a major pathway in UV light induced carcinogenesis, we designed a study in cultured human prostate cancer cells to unravel the protective properties of selenium towards DNA. We thus studied the protection afforded by two different selenium compounds, sodium selenite (SS) and selenomethionine (SM), against cytotoxicity, induction of UV light induced-DNA damage and modulation of DNA repair activities. Firstly we found that pretreatment of the human prostate cancer cells (LNCaP) with 30 nM of SS and 10 µM of SM for 72 h protects from cell death induced by UVA radiation, a treatment associated with extensive oxidative stress. This results correlate with the extent of DNA damage and protection observed for the different toxics and revealed by the Comet assay. We also determined that Se enhances the DNA repair capacities in cells. This trend was most significant for UVA than UVC-induced damage.

This project will allow us to a better understanding of mechanisms by which selenium could protect human against cancer.

ALA dendrimers to improve diagnostic specificity of fluorescence guided diagnosis of bladder cancer

A François[1], L Bezdetnaya[1], S Battah[2,3], F Guillemin[1], A MacRobert[3], M D'Hallewin[1]
[1]*Centre de Recherche en Automatique de Nancy, CNRS, Nancy University, Centre Alexis Vautrin*
[2]*National Medical Laser Centre, UCL Medical School, University College London, The Laser Centre and School of Biological Sciences, University of Essex, UK*
[3]*National Medical Laser Centre, UCL Medical School, University College London, UK*

Background: Fluorescence guided cystoscopy with 5-aminolevulinic acid (ALA) or its hexyl derivative (h-ALA, Hexvix®) has been shown to increase bladder cancer diagnosis and offer the possibility to perform a more complete resection, thereby reducing the recurrence and progression rate. The fast photobleaching of the fluorophore protoporphyrin IX (PpIX) limits the visualization during cystoscopy and the specificity is low.

Methods: To overcome these drawbacks, dendrimers containing 18 ALA molecules (18-ALA) were synthesized. We investigated the *in vitro* hydrolysis of ALA, the PpIX synthesis as well as the photobleaching of equimolar concentrations of ALA, h-ALA and 18-ALA. We instilled 18-ALA intravesically in rats, bearing orthotopic bladder tumors to study the specificity on frozen sections by fluorescence microscopy.

Results: A slow mono-exponential hydrolysis of the dendrimers probably attributed to steric hindrance and limited access to esterases is observed, as opposed to the rather fast liberation of free ALA from Hexvix®. The continuous synthesis of PpIX and the slower photobleaching of dendrimer induced PpIX, would thus ensure a prolonged presence of fluorescence during cystoscopy. *In vivo*, 1h instillation is sufficient to enable endocytosis of dendrimers but a minimum of 3h resting time is required to observe PpIX fluorescence with a better specificty towards the tumor as opposed to h-ALA.

Conclusion: The prolonged and sustained PpIX synthesis with a reduced photobleaching and the improved specificity are primary indicators that ALA dendrimers could possibly overcome drawbacks of ALA/h-ALA fluorescence guided cystoscopy.

Battah S, O'Neil S, Edwards C, Balaratnam S, DobbinP, MacRobert AJ. Enhanced porphyrin accumulation using dendritic derivatives of 5- aminolevulinic acid for photodynamic therapy: an in vitro study. Int J Biochem Cell Biol 2006, 38:1382-92

2. Battah S, Balaratnam S, Casas A, O'Neil S, Edwards C, Batlle A, Dobbin P, MacRobert AJ. Macromelecular delivery of 5- aminolevulinic acid for photodynamic therapy using dendrimer conjugates. Mol. Cancer Ther. 2007, 6, 876-885.
3. Casas A, Battah S, Di Venosa G, Dobbin P, Rodriguez L, Fakuda H, BatlleA, MacRobert AJ. Sustained and efficient porphyrin generation in vivo using dendrimer conjugates of 5-ALA for photodynamic therapy. J. Control. Release, 2009, 135:136-143.
4. Iinuma S, Bachor R, Flotte T, Hasan T. Biodistribution and phototoxicity 5-ALA induced PpIX in an orthotopic rat bladder model. J Urol 1195,153,802-6.

Spatio-temporal redistribution of mTHPC from lipid nanovesicles in chick chorioallantoic membrane model

J.Garrier[1], V.Reshetov[1,3], D.Dumas[2], V.Zorin[3], A.François[1], MA. D'Hallewin[1], F.Guillemin[1], L.Bezdetnaya[1]

[1]Centre de Recherche en Automatique de Nancy (CRAN), Nancy-University, CNRS, Centre Alexis Vautrin, Vandœuvre-lès-Nancy, France. [2]LEMTA, Equipe Mécanique et Ingénierie Cellulaire et Tissulaire, Vandœuvre-lès-Nancy, France. [3]Laboratory of Biophysics and Biotechnology, Belorussian State University, Minsk, Belorussia.

Liposomal formulations of mTHPC (Foslip®, Fospeg®) were initially proposed to improve its chemico-biological properties and distribution in targeted tissues. They permitted the preservation of the monomeric state of the molecule and the enhancement of its pharmacokinetic and clearance. Previous experiments realized by our laboratory with mTHPC (Mitra *et al*. 2005, Garrier *et al*. 2010; Lassalle et al 2009) demonstrated the primary importance of spatio-temporal distribution of the molecule for optimization of PDT dosimetric parameters (drug-light interval, drug dose…).

Optimization of PDT parameters for photosensitizers embedded into the liposomes also requires the comprehension of the PSs repartition from lipid nanovesicles. However, there are not data concerning the distribution of mTHPC in relation with the degradation of lipid nanovesicules administered *in vivo*. This analysis is actually conducted by using the chick chorioallantoic membrane (CAM) model with lipid nanovesicles which contain mTHPC and a fluorescent probe of nanovesicle integrity pyrene. Activity of pyrene is based on a mechanism of FRET (Fluorescence Resonance Energy Transfer) where pyrene plays the role of energy donor while mTHPC stands for an acceptor of energy. The measurement of their fluorescence lifetimes by FLIM technique (Fluorescence Lifetime Imaging Microscopy) reflects the state of integrity of the lipid nanovesicule. The energy transfer in intact liposome was confirmed by a strong diminution (>1000 fold) of fluorescence lifetime of pyrene in the presence of mTHPC. The optimization of this technique permits to study fluorescence lifetime in CAM in relation with the time after intravenous injection of mTHPC loaded in liposome.

- Mitra S, Maugain E, Bolotine L, Guillemin F, Foster TH. Temporally and spatially heterogeneous distribution of mTHPC in a murine tumor observed by two-color confocal fluorescence imaging and spectroscopy in a whole mount model. Photochem. Photobiol. 2005;81:1123-1130

- Garrier J. Bressenot A., Gräfe S., Marchal S., Mitra S. Foster TH., Guillemin F., Bezdetnaya L. Compartmental targeting for mTHPC based photodynamic treatment in vivo: correlation between efficency, pharmacokinetics and regional distribution of apoptosis. Int. J. Radiat. Oncol. Biol. Phys. 2010. In press

- Lassalle HP, D. Dumas, S. Gräfe, M-A. D'Hallewin, F. Guillemin, L. Bezdetnaya. Correlation between *in vivo* pharmacokinetics, intratumoral distribution and photodynamic efficiency of liposomal mTHPC . J Control Release, 2009;134:118-24

Effects of UVA-induced oxidative stress on DNA replication in mammalian cells

Dany Graindorge[a,b], Pierre-Marie Girard[a,b], Evelyne Sage[a,b].

[a] CNRS UMR3348, Centre Universitaire, 91405 Orsay, France

[b] Institut Curie, Bât. 110, Centre Universitaire, 91405 Orsay, France

The amount of Ultraviolet A (UVA) radiation reaching the skin is approximately 20 times greater than that of UVB radiation. Although the carcinogenic effect of UVB is well established, several reports indicate that UVA radiation plays also an important role in the pathogenesis of photodermatosis, photoaging and melanoma skin cancer [1]. Exposure to UVA induces the formation of photoexcited states of cellular photosensitizers with subsequent generation of reactive oxygen species (ROS) leading to damages to membrane lipids, proteins and nucleic acids [2]. Although UVA radiation, unlike UVC and UVB, is poorly absorbed by DNA, it inhibits cell cycle progression in a dose-dependent manner, especially during S-phase [3, 4].

Our aim is to determine which mechanism governs this UVA-induced slowdown of DNA replication. To do so, we investigate the effects of UVA on the DNA replication initiation and elongation in mammalian cells using molecular combing. More precisely, this technique will allow us to know if UVA radiation influences the origin firing and/or the fork progression. Moreover, as this delay could be the consequence of UVA-induced depletion or oxidation of deoxyribonyucleoside triphosphate (dNTP) pools, a quantification of the intracellular level of each dNTP after UVA, using an enzymatic assay, is also under investigation. Our preliminary data show i) a decrease of replication fork velocity and ii) a drop in dTTP and dATP levels after UVA irradiation. Based on these results, it is important to determine whether the slowdown of DNA replication is a consequence or not of the low level of dNTP observed post UVA.

[1]. Krutmann J. Ultraviolet A radiation-induced biological effects in human skin: relevance for photoaging and photodermatosis. *J Dermatol Sci*, vol. 23, Suppl 1, S22-26, 2000.

[2]. Wondrak GT, Jacobson MK, Jacobson EL. Endogenous UVA-photosensitizers: mediators of skin photodamage and novel targets for skin photoprotection. *Photochem Photobiol Sci,* vol.5, 215-237, 2006.

[3]. Bånrud H, Stokke T, Moan J, Berg K. S phase arrest and induction of multinucleated cells after exposure to ultraviolet radiation. Carcinogenesis, vol.5, 1087-94, 1995.

[4]. Girard PM, Pozzebon M, Delacôte F, Douki T, Smirnova V, Sage E. Inhibition of S-phase progression triggered by UVA-induced ROS does not require a functional DNA damage checkpoint response in mammalian cells. DNA Repair, vol.9, 1500-16, 2008.

The biosynthesis of flavonoidsis enhnced similarly by UV-radiation and root-zone salinityin l. Vulgare leaves

Guidi L.[*], Degl'Innocenti E.[*], Tattini M.[**]
[*] *Department of Plant Biology, University of Pisa, Pisa, Italy,* [**] *Institute for Plant Protection, IPP, CNR, Sesto Fiorentino, Florence, Italy*

Flavonoids have been recently reported as having the potential to serve multiple functions in photoprotection, possibly as a consequence of their antioxidant features. Here the hypothesis was tested that flavonoids accumulate in response to excess-light, in the presence or in the absence of UV-radiation. In an UV-exclusion experiment, we grew *Ligustrum vulgare* plants outdoors, under 30 or 100% sunlight irradiance, by cutting-off the UV-waveband. These plants were additionally exposed to UV-irradiance or supplied with 125 mM NaCl at the root-zone. Leaves of plants under 100% sunlight irradiance actually suffered from excess light, which was exacerbated greatly by root-zone salinity stress. Salinity stress repressed the activities of antioxidant enzymes, particularly in full sunlight, and enhanced the leaf oxidative damage. Dihydroxy-B-ring-substituted flavonoids, namely quercetin-3-O and luteolin 7-O-glycosides, accumulated steeply in response to sunlight irradiance, in the absence of UV-radiation. UV-radiation and root-zone NaCl increased to a very similar degree the concentration of these flavonoids, which are effective in scavenging various reactive oxygen form. Actually, treatment-induced changes in leaf phenylpropanoid concentration mostly affected the antioxidant activities, not the UV-screening capacities of leaf extracts. Early responses to an abrupt increase in sunlight irradiance included a steep increase in quercetin derivatrive and anthocyanin (which negligibly absorb in the UV-spectral region) concentrations, whereas effective UV-attenuators, like hydroxycinnamates and monohydroxy B-ring flavonoids, were unresponsive to the light-treatments. Overall, these findings lead to the hypothesis og key antioxidant functions of flavonoids in photoprotection. This hypothesis is further corroborated by the large distribution of quercetin and luteolin in the vacuoles of mesophyll, not only in the corresponding compartments of epidermal cells, in full sunlight-treated leaves in the absence of UV-radiation.

Biodistribution of near-infrared emitting CuInS2/ZnS quantum dots by mass spectroscopy and fluorescence imaging of axillary lymph node in healthy mice

Marion Helle, Emilie Pic, Thomas Pons, Lina Bezdetnaya, François Guillemin, Benoit Dubertret, Frédéric Marchal
CRAN, Nancy-University, CNRS, Centre Alexis Vautrin, Avenue de Bourgogne, 54511 Vandoeuvre-lès-Nancy Cedex, France
Laboratoire Physique et Etude des Matériaux, CNRS UPR0005, ESPCI, 10, rue Vauquelin, 75005 Paris, France.

E-mail: m.helle@nancy.fnclcc.fr

Background : The biopsy of the sentinel lymph node is now widely used for the breast cancer. Although this approach has strong advantages, it has its own limitations (manipulation of radioactive products and possible anaphylactic reactions to the dye). As recently proposed, these limitations could in principle be by-passed if semiconductor nanoparticles (quantum dots or QDs) were used as fluorescent contrast agents for the in vivo imaging of sentinel lymph nodes. QDs are fluorescent nanoparticles with unique optical properties like strong resistance to photobleaching, size dependent emission wavelength, large molar extinction coefficient, and good quantum yield.
Materiel and Methods : 20 µL of 1 µM CuInS2/ZnS core/shell QD solution were injected subcutaneously in the right anterior paw of healthy balb/c mice. Animals were sacrificed at different time points after QDs injection. Organs, blood and excretions were collected and analysed by ICP-MS. RALNs and RLTLNs were removed and weighed for histological analysis to visualize inflammatory changes.
Results : CuInS2/ZnS QDs were observed in RALN, studied organs and blood as soon as 5 min and up to 10 days after the injection by ICP-MS and in vivo fluorescence imaging. The maximum amount of QDs in the ALN was detected 24 h after injection and corresponded to 5 % of the injected dose Histological sections of RALNs dissected 7 days post-injection have shown that the RALN of mice injected with 20 pmol of CuInS2/ZnS showed no appreciable difference with control LNs.
Conclusion : Effective and rapid detection of ALN using fluorescence imaging of NIR emitting CuInS2/ZnS QDs in living animals was demonstrated. Nevertheless, the improvement of their surface chemistry are required to allow their excretion, and therefore, to eliminate any risk of toxicity.

Cadmium-free CuInS2/ZnS quantum dots for sentinel lymph node imaging with reduced toxicity. Pons T, Pic E, Lequeux N, Cassette E, Bezdetnaya L, Guillemin F, Marchal F, Dubertret B. ACS Nano. 2010 May 25;4(5):2531-8.

Optimizing PDT by Liposomal formulation of the photosensitizer PPME: Comparative biophysical investigations in HCT 116 cells.

Maryse Hoebeke[a], C. Quoilin[a], Angeliki Grammenos[a], Sandrine Lécart[c],Jacques Piette[d], Marie-Pierre Fontaine-Aupart[b] and Pierre-Henri Guelluy[a]

a Biomedical Spectroscopy, Department of Physics, University of Liège, Liège, Belgium,
b Institut des Sciences Moleculaires d'Orsay, FRE 3363, University of Paris-Sud 91405 orsay, France - CNRS 91405 Orsay, France
c CPBM / LUMAT, FR 2764, University of Paris-Sud 91405 Orsay, France
d GIGA-R: Virology and Immunology, B34, University of Liège, B-4000 Liège, Belgium

Photodynamic therapy (PDT), induced by photosensitizer (PS) encapsulated in nanostructures, emerges as an appropriated treatment to cure a multitude of oncological and non-oncological diseases. Pyropheophorbide-a-methyl ester (PPME) is a second-generation PS tested in PDT and a potential candidate for future clinical applications. The present study, carried out in a human colon carcinoma cells (HCT-116), evaluates the improvement brought by a liposomal formulation of PPME versus free-PPME. bsorption and fluorescence spectroscopies, fluorescence lifetime measurements, subcellular imaging and co-localization analysis have been performed in order to analyze the properties of PPME for every delivery modes. The benefit of drug encapsulation in DMPC-liposomes appears clearly in our experiments with a 5-fold higher intracellular drug delivery than observed with free-PPME at similar concentrations and the consequently increase of irradiated cells death. The reactive oxygen species (ROS) produced after PPME mediated photosensitization have been identified and quantified by using electron spin resonance (ESR). Irradiation of the dye in HCT 116 cells in the presence of POBN spin trap and ethanol scavenger (2%, a non toxic concentration) leads to the apparition of the ESR spectrum characteristics of POBN-ethoxy adduct. Addition of specific ROS quenchers like catalase, SOS, DABCO allows us to show that PPME is able to generate superoxide anions, hydroxyl radicals and singlet oxygen. The parallel effect of SOD and catalase suggests that superoxide anion and hydrogen peroxide are involved in the generation of hydroxyl radical via a Fenton reaction. The comparison of ESR spectra obtained with free PPME and with lipo-PPME reveals an increase of the POBN /ethoxy spin adduct signal for an equivalent concentration of drug, consequence of a better intracellularization of PPME provided by the drug vectorization. The weak presence of PPME inside mitochondria, as revealed by co-localization analysis, probably explained the very low apoptotic cell death measured in HCT-116.

Reference: Optimizing Photodynamic Therapy by Liposomal formulation of the photosensitizer Pyropheophorbide-a-methyl ester: *In vitro* comparative biophysical investigations in a colon carcinoma cell line. Pierre-Henri Guelluy, Marie-Pierre Fontaine-Aupart , Angeliki Grammenos, Sandrine Lécart, Jacques Piette and Maryse Hoebeke , Photochemical & Photobiological Sciences, in press

Effect of cholesterol on the mechanisms involved in photo-permeabilization of membrane.

Rachid Kerdous, Stéphanie Bonneau

Laboratoire ANBiopHy, CNRS FRE3207, Université Pierre et Marie Curie, Paris

The Photochemical Internalization (PCI) is a method allowing the photo-release of macromolecules trapped in endosomes after endocytosis. It is based on the ability of certain photosensitizers to localize preferentially within the endosomal membranes and to produce reactive oxygen species (ROS) under light irradiation. The subsequent oxidation of the surrounding unsaturated lipids induces the permeabilization of the membrane of endosomes.

Cholesterol plays an important role in biological membranes, in particular in endosomale ones: the cholesterol content of the membranes profoundly modulates their physical, structural and dynamic properties. It represents approximately 30% of the total lipid mass of endosomes and its physiological importance has been widely demonstrated. However, its effects during PCI remains poorly understood. In order to apprehend this question, we used giant unilamellar vesicles (GUVs) as membrane models prepared from a series of mixtures of DOPC and cholesterol (0, 10, 20, 30 and 50 mol%). Photopermeabilization experiments were conducted using the chlorine e6, which cannot cross the bilayer and will then mark the membrane asymmetrically.

Following light irradiation, the membranes fluctuate during a period ranging from several seconds to several hundreds of seconds. Fluctuations are followed by changes in membrane morphology. These shape transitions are explained by changes in the effective spontaneous curvature of the marked membrane monolayer and interpreted regarding the amount of cholesterol in the membrane. Notably, the duration of the fluctuations is inversely proportional to its concentration. Cholesterol also affects strongly time to start (t_0) and the speed of membrane permeabilization (τ).

References

Julien Heuvingh, Stéphanie Bonneau. Biophysical Journal, december 2009, 97(11):2904-2912.

Halina Mojzisova, Stéphanie Bonneau, Philippe Maillard, Kristian Berg and Daniel Brault. Photochemical & Photobiological Sciences, june 2009, 8(6):778-87.

Halina Mojzisova, Stéphanie Bonneau, Christine Vever-Bizet, Daniel Brault. Biochimica et Biophysica Acta - BBA (Biomembranes), november 2007, 1768(11):2748-56.

Supercontinuum for Foerster resonance energy transfer and lifetime imaging applications.

Raffaella Mercatelli[1], Silvia Soria[2*], Franco Quercioli[3*], Giancarlo Righini[2].
[1] *Dipartimento di Fisiopatologia clinica, Viale Pieraccini 6, 50139 Firenze, Italy*
[2] *IFAC-CNR, Istituto di Fisica Applicata "N. Carrara", Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy*
[3] *ISC-CNR, Istituto dei Sistemi Complessi, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy*

The study of protein dynamics in living cells is of crucial importance in biology and medicine since protein-protein interactions mediate several cellular processes. Imaging with high spatial and temporal resolutions is required to study such processes.
Hyperspectral FLIM [1] is a technique allowing simultaneously recording of the full emission spectrum and temporal decay curves of a biological specimen and may be useful for Foester resonance energy transfer (FRET) measurements [2]. FRET between two fluorescent labels, donor and acceptor, is a well tested spectroscopic technique for measuring distances below 10 nm, which is the order of the distance between proteins bound in a complex [3].
A critical component for a fluorescence microscope is still the excitation source. FLIM requires an ultrafast excitation source and this can be provided by supercontinuum (SC) generation in microstructured fibres (MOF). SC provides the capability of simultaneous optical imaging and spectroscopy with multiple wavelengths to characterise biological samples. Developing filtering techniques in order to select discrete wavelengths from the SC source has always been of interest.
In this work, we have filtered the broadband SC generated from a commercial MOF by means of a spectroscope lens, specially designed based on longitudinal chromatic aberration [4]. The intentionally generated aberration has been used as an useful on-axis spectroscope for resolving the excitation source in a range from 400 to 1000 nm with a width similar to an interferometric filter. The spectroscope lens is simple, tight and accurate; it does not require neither a collimated beam to work with nor a special steady mount. We have validated our spectroscope lens by measuring hyperspectral FLIM-FRET *in vivo* human embryonic kidney cells in order to characterize the interaction between two cellular membrane proteins, a potassium channel and integrin-beta1, stained, respectively, with enhanced Cyan Fluorescence Protein (ECFP) and enhanced Yellow Fluorescent Protein (EYFP).

References: **1)** French "Excitation-resolved hyperspectral fluorescence lifetime imaging using a UV-extended supercontinuum source" Opt. Lett. **32**, 3408-3410 (2007) **2)** E.A. Jares-Erijman and T.M. Jovin, "Imaging molecular interactions in living cells by FRET microscopy" Curr. Opin. CHem. Biol. **10**, 409-416 (2006) **3)** J.R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd Edition (Springer, US, 2006) **4)** F. Quercioli, B. Tiribilli, G. Molesini, "Optical Surface profile transducer" Opt. Eng.27, 135-142 (1988);

Ultrafast photoactivation of two new photolyase/cryptochrome proteins in their oxidized state

J. Brazard[1], A. Usman[1], F. Lacombat[1], C. Ley[1], M. M. Martin[1], P. Plaza[1], L. Mony[2], M. Heijde[3], G. Zabulon[3], C. Bowler[3]
1 UMR 8640, Département de Chimie, Ecole Normale Supérieure, 75005 Paris, France
2 UMR 8601, Université Paris Descartes, 75006 Paris, France
3 UMR 8186, Département de Biologie, Ecole Normale Supérieure, 75005 Paris, France

The flavin adenine dinucleotide (FAD) cofactor of cryptochromes and photolyases is known to be reduced to an active state by irradiation in the visible domain [1]. For *E. coli* CPD photolyase bearing the flavin in its semi-reduced form ($FADH^{\bullet}$), this photoactivation reaction was proposed to occur through a sequential electron transfer along a chain of three conserved tryptophan residues [2]. The rate limiting step of the whole process was however the primary photoreduction of the flavin in ~30 ps. Two new members of the cryptochrome/photolyase family, belonging to the green alga *Ostreococcus tauri*, were recently discovered (OtCPF1 and OtCPF2) [3]. We studied the photoactivation dynamics of these proteins, bearing the oxidized form of the flavin (FAD_{ox}), by broadband UV-Vis femtosecond absorption spectroscopy. We observed in both cases the ultrafast photoreduction of FAD_{ox}, in 390 fs for OtCPF1 and 590 fs for OtCPF2, and made a full spectral characterization of the reaction. The analysis of the photoproduct spectra allowed identifying tryptophan as the primary electron donor. This residue is found to be oxidized to its protonated radical cation form ($WH^{\bullet+}$), while FAD_{ox} is reduced to $FAD^{\bullet-}$. Subsequent kinetics were observed in the ps and sub-ns regime, mostly described by a biexponential partial decay of the photoproduct transient signal (9 and 81 ps for OtCPF1, and 13 and 340 ps for OtCPF2), while a long-lived photoproduct remains in the ns timescale. We interpret these observations within the Brettel and Vos model [2]. By direct excitation of the cofactor in its oxidized form FAD_{ox} the present study permitted to reveal the kinetic steps following the initial photoreduction of the flavin and spectroscopically assign them to the hole hopping process along the tryptophan chain, accompanied by partial charge recombination at each step [4]. Preliminary transient anisotropy measurements performed on OtCPF2 support this mechanism.

References
[1] A. Sancar, Chem. Rev. 103, 2203 (2003).
[2] M. Byrdin, A. Lukacs, V. Thiagarajan, A. Eker, K. Brettel, M. Vos, J. Phys. Chem. A 114, 3207 (2010).
[3] M. Heijde, G. Zabulon, F. Corellou , T. Ishikawa , J. Brazard , A. Usman, F. Sanchez, P. Plaza, M. Martin, A. Falciatore, T. Todo, F.-Y. Bouget, C. Bowler, Plant, Cel & Env. Available online, doi: 10.1111/j.1365-3040.2010.02168.x (2010).
[4] J. Brazard, A. Usman, F. Lacombat, C. Ley, M. Martin, P. Plaza, L. Mony, M. Heijde, G. Zabulon, C. Bowler, J. Am. Chem. Soc. 132, 4935 (2010).

Photaddition of a ruthenium complex(II) on tryptophan. Determination of the photoadducts structure

Virginie Rahal[1], Jean Christophe Garrigue[1], Daniel Brault[2], Patricia Vicendo[1]
1- Laboratoire des IMRCP, UMR-CNRS 5623, Université Paul Sabatier, 31062 Toulouse Cedex 07
2- UPMC – Paris 6, Laboratoire ANBioPhy CNRS FRE3207, Case courrier 138, 4 place Jussieu, 75252 Paris Cedex 05, Paris

Due to their photoredox properties, ruthenium (II) complexes can react with biological targets by different photochemical mechanisms (type I, type II). Depending on their ligands, it will be possible to modulate their photo-reactivity. Thus when the target is DNA, these compounds may find numerous applications such as photonucleases, alkylating agents of DNA. In previous work, we demonstrated that some of these compounds that are highly photo-oxidant, could also react with proteins such as the superoxide dismutase Cu / Zn via a complex mechanism involving two successive electron transfer processes. This original reaction would involve an amino acid with a low redox potential, located on the surface of the protein, such as tryptophan. To confirm this hypothesis we studied the photosensitisation of tryptophan, tyrosine and of a tripeptide glu-trp-glu by ruthenium (II) complexes. By EPR studies and flash photolysis investigations, we have shown the formation of the transient species Ru1+ and the tryptophan radical cation upon irradiation of trisbipyrazine ruthenium (II) complex (Ru(bpz)32+), in the presence of tryptophan and tripeptide. Ru(bpz)32+, which is a strong photo-oxydizer, may at the excited state oxidize tryptophan via an electron transfer process. The photoproducts generated during this reaction were analyzed by mass spectrometry. This study revealed the formation of photoadducts between the tryptophan and Ru(bpz)32+. The structures of these photoadducts were clearly identified and will be presented. This result tends to prove that the formation of photoadducts [ruthenium complex-tryptophan] on the SOD might be partly responsible of the enzyme inhibition. This study will have interesting applications in the development of new photo-activable agents for photodynamic therapy.

Interaction of mTHPC liposomal formulations with serum proteins

Vadim Reshetov1,2, Vladimir Zorin2, Lina Bolotine1
1 Centre de Recherche en Automatique de Nancy, Nancy-Université, CNRS, Centre Alexis Vautrin, Vandœuvre-Les-Nancy, France
2 Laboratory of Biophysics and Biotechnology, Physics Faculty, Belarusian State University, Minsk, Belarus

The use of liposomes as drug delivery systems is an accepted approach to improve the photosensitizer efficacy. Because of their characteristic small size (between 50 and 200 nm) and good solubility and stability, liposomes represent an ideal delivery system for nonpolar photodrugs. In this perspective, a clinically approved photosensitizer, meta-tetra(hydroxyphenyl)chlorin (mTHPC) has been loaded into liposomes with or without addition of PEGylated lipid. The present study addresses the distribution pattern of liposomal mTHPC (Foslip® and Fospeg®) in blood serum assessed by gel-filtration chromatography

It was found that the affinity of pure and liposome-based mTHPC towards different plasma proteins is almost identical. The major part of the photosensitizer localizes in the high density lipoproteins fractions, while a minor fraction passes through the column with low-density lipoproteins. Only a small part of mTHPC molecules is found in the albumin fraction.

As opposed to conventional liposomes with a very rapid disruption of the lipid vesicles and fast release of the drugs, mTHPC loaded DPPC/DPPG liposomes show a very slow release of the active component. After 30 min of Foslip® incubation with serum only a small percentage of mTHPC redistributes from the liposomes. Increasing incubation time to 6 h results in a significant reduction of the mTHPC fluorescence signal associated with mTHPC embedded into liposomes and concomitant increase of the signal associated with the protein-based bands. After 24 h incubation the distribution pattern was similar to the elution profile of serum containing free mTHPC.

In contrast to Foslip®, short incubation of PEGylated liposomes containing mTHPC (Fospeg®) with serum results in a release of approximately half of mTHPC from the lipid carriers. The kinetics of release is clearly two-phased: rapid release followed by slow redistribution. The slow phase is decelerated compared to Foslip®.

Optical detection of gold nanorods by darkfield spectroscopy and imaging: preliminary applications to biological systems.

Giovanni Romano[1], Raffaella Mercatelli[1], Fulvio Ratto[2], Sonia Centi[1], Franco Fusi[1]

[1]Medical Physics section, Department of Clinical Patophysiology, viale Pieraccini 6 – 50135 Florence

[2]IFAC – CNR, via Madonna del Piano 10, 50019 Sesto Fiorentino (Florence)

In recent years, the development of new solutions for the production and functionalization of metal and gold nanoparticles (GNPs), has been accompanied by a growing interest in the investigation of their optical properties, which are at the basis of many important applications of GNPs in the medical field.

One of the most promising optical techniques employed to study GNPs localization and interaction with a biological sample (e.g. a cell culture) is darkfield microscopy (DM), which takes advantage of the peculiar light scattering properties of GNPs to localize them within a polymer gel or a cell culture sample. In particular, DM produces highly contrasted images by acquiring the light scattered by the sample in opportunely chosen spectral bands: one corresponding to the GNPs scattering peak, the other centered in the illumination light spectrum peak (e.g. halogen illumination).

By a division operation between the pixel matrixes corresponding to the two images, constrast is achieved thanks to the very different ratio between scattered light peaks in the case of GNPs respect to the rest of the sample. Image acquisition can be accompanied by the measurement of the absorption spectrum of GNPs, both with and without the presence of other scatterers such as cell organelles or polymer agglomerates. The fingerprint of GNPs presence is represented by a double-peaked scattering spectrum, whose shape depends mainly on GNPs geometry.

By combining the two techniques (optical imaging and spectral measurements) we have been able to localize and detect GNPs included in polymer matrixes and glass-adherent cell cultures, posing the basis for further study of their interaction with biological systems at the cellular scale.

The photodynamic effect of tetra-substituted *N*-methyl-pyridyl-porphine combined with the action of vancomycin or host defense mechanisms disrupts Staphylococcus epidermidis biofilms

Enrica Saino[1,2], Antonella Di Poto[1,2], Mariangela Scavone[1,2], Nora Bloise[1,2], Livia Visai[1,2]

[1]Department of Biochemistry, University of Pavia, Pavia – Italy; [2]Center for Tissue Engineering (CIT), University of Pavia, Pavia - Italy (1 riga vuota; sempre a spaziatura 1)

The skin bacterium *Staphylococcus epidermidis*, once considered harmless, is the leading cause of nosocomial infections, especially in patients with predisposing factors such as indwelling or implanted foreign polymer bodies. Virulence is attributable to formation of biofilm, which provides a microenvironment that protects the bacterium from attack by the host immune system and by chemotherapy. Photodynamic treatment (PDT) is a process in which microorganisms are treated with a photosensitizer drug and then with low intensity visible light of the appropriate wavelength. Recently, we demonstrated a significant inactivation of *S. epidermidis* cells by exposing bacteria to toluidine blue O (TBO) and laser simultaneously (1). We also demonstrated that pretreatment of *S. aureus* biofilms with N-methyl-pyridyl- porphine (TMP) based-PDT, followed by either addition of vancomycin at concentrations significantly below the biofilm inhibitory concentration values or by exposure of disrupted cell clusters to phagocytosis, causes disruption of the biofilm matrix and results in almost complete killing of bacteria (2).

In this study we extended to *S. epidermidis* strategies previously aimed at treatment of *S. aureus* biofilms using photodynamic treatment (PDT) combined with chemotherapy or phagocytosis. A significant reduction in bacterial survival was observed when structurally distinct biofilms were exposed to the cationic porphyrin, tetra-substituted N-methyl-pyridyl-porphine (TMP), and simultaneously to visible light. Of note, the extent of biofilm clearance depended on its maturation stage: developing, young biofilms, were more sensitive towards PDT than mature biofilms. Furthermore, PDT-treated biofilms exposed to vancomycin or subjected to phagocytic action of whole blood were almost completely eradicated. The data we obtained establish that PDT combined with antibiotics or host defenses may also be a useful approach for the inactivation of *S. epidermidis* biofilms.

References: **1.** Sharma M, *et al.* Antimicrob Agents Chemother 2008; 52: 299-305; **2.** Di Poto A, *et al.* Biomaterials 2009; 30: 3158-66.

Hypericin incorporation in fixed HeLa cells

T. K. T. Vuong, C. Vever-Bizet, S. Bonneau and G. Bourg-Heckly
Laboratoire ANBiophy, CNRS FRE 3207, Université Pierre et Marie Curie, Paris

Cervical cancer is the second most common cancer and third cause of cancer death among women.The organization of screening programs based on cytological testing commonly called Papanicolaou smear has greatly reduced the invasive cervical cancer incidence and mortality. The detection and grading of cancers by this method are based on several morphological anomalies of cervical cells preserved in a fixative. Despite the effectiveness of the method, its sensitivity remains unsatisfactory (from 40 to 86%) [1].

Hypericin is a photosensitizer expressing high affinity for cancerous cells *in vivo*. Diagnosis of cancer based on hypericin fluorescence imaging has been successfully assessed in several clinical trials (sensitivity ranges from 82% to 94% and specificity from 91% to 98.5%) [2, 3]. The majority of these studies were carried out *in vivo* and some *ex vivo* studies were reported but exclusively on live cells.

Our objective is to evaluate the potential of hypericin fluorescence imaging to improve the efficacy of cervical cancer diagnosis performed on fixed cell smears obtained from liquid-based cytology.

For this purpose, the mechanism of hypericin incorporation and localization in fixed HeLa cells using different incubation media and fixation conditions was investigated. Since the duration of fixation may play an important role, the influence of fixation time on hypericin incorporation in fixed HeLa cells was studied. The uptake and distribution of hypericin in fixed HeLa cells were found strongly dependent on the hypericin incubation medium: for a polar organic solvent such as the alcohol-based fixative, the localization was essentially perinuclear and nuclear; for cell culture medium supplemented with serum, the localization was cytoplasmic and non-specific; the highest incorporation was observed for the culture medium serum free but mainly as non-fluorescent aggregates. The hypericin aggregation in the incubation medium, the passive diffusion and the partitioning between the cells and hypericin carriers seemed to be the major factors accounting for these results. The localization was found weakly dependent on fixation time whereas fluctuations of hypericin fluorescence at short fixation time and stabilization after two days of fixation were observed.

References

1. *International Agency for Research on Cancer, Lyon: IARC Press*, 2005, **10**, 1–302.
2. M. A. D'Hallewin, A. R. Kamuhabwa, T. Roskams, P. A. M. De Witte and L. Baert, *BJU Int.*, 2002, **89**, 760-763.
3. D. Jocham, H. Stepp and R. Waidelich, *Eur. Urol*., 2008, **53**, 1138-1150.

Actinic keratoses photodynamic treatment: MAL-PDT vs ALA-PDT, a retrospective study

Zanieri F., Rossi R., Mori M., Urpe* M., Bonciani D., Lotti T
*Dermatological clinic II of Department of Medical and Surgical Critic Area, * Interuniversity Centre of Biologic and Psychosomatic Dermatology (CIDEBIP), University of Florence, Florence, Italy.*

Actinic keratoses (AK) is a common keratinocyte intraepithelial neoplasia i.e. a squamous cell carcinoma in situ. Thus AK must be treated and removed using one of several different methods of therapy selected by the dermatologist. It has been calculated that 60% of subjects with fair skin (from I to III phototype) over 40ies present at least one AK. This diffuse and often progressing condition (towards are invasive squamous cell carcinoma) requires a strategy of prevention, a prompt diagnosis and an adequate treatment. Photodynamic therapy using topical photosensitizer has been demonstrated to be useful in the treatment of various non-melanoma skin cancer (approved in Europe for the treatment of superficial and nodular basal cell carcinoma, Bowen's disease and AK). Aks are the most common dermatological indications for PDT.

In our retrospective study, the results obtained by using two different photosensitizers, 5-aminolevulinic acid (ALA) and its methyl esther (methylaminolevulinate-MAL), have been compared. Our protocol included 152 patients (106 treated with MAL-PDT and 46 with ALA-PDT and we divided the body in great areas (head, trunk and arms) analysing the results for each area. Our results showed that MAL is more efficient than ALA in treating AKs and considering the global costs MAL-PDT is less expensive than ALA-PDT. At today no comparative studies are present in the literature.

References

1. Rossi R, Mavilia L, Ghersetich I, Lotti T. Photodynamic therapy of actinic keratoses with methyl-aminolevulinate (METVIX®). G Ital Dermatol Venereol 2005;140:325-327.
2. Rossi R, Lotti T, Cappugi P., Sala R, Venturini M, Calzavara-Pinton P.G., and GIDFE. Guidelines for photodynamic therapy in dermatology: treatment protocol. G Ital Dermatol Venereol, 2005; 140: 637-44.
3. Rossi R, Mori M, Lotti T. Actinic keratosis. Int J Dermatol. 2007 Sep;46(9):895-904.
4. Rossi R, Assad GB, Buggiani G., Lotti T. Photodynamic therapy: treatment of choise for actinic cheilitis. Dermatologic Therapy 2008, Sept-Oct;21(5):412-5.
5. Rossi R., Calzavara-Pinton PG, Giannetti A., Peserico A., Santucci M., Vena GA, Lotti T. Italian guidelines and therapeutic algorithm for actinic keratoses. G Ital Dermatol Venerol. 2009 Dec;144(6):713-23.

Fractional CO_2 laser: clinical studies and non-invasive investigations

Zanieri F., Campolmi P., Bonan P., Cannarozzo G., Troiano M., °Morini C., *Pavone F. S., Lotti T.

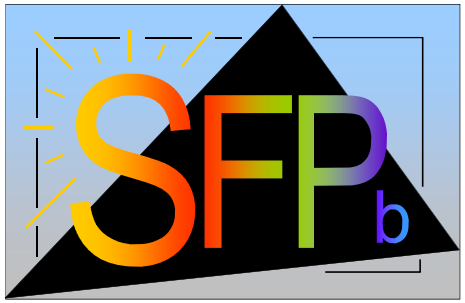
*Department of Critical Medicine and Surgery, Dermatologic Second Clinic, University of Florence; °Interuniversity Centre of Biological and Psychosom atic Dermatology (CIDEBIP); *Department of Physic and Astronomy, University of Florence.*

Recent developments in the search for innovative laser treatment methods have given birth to fractional systems, and in particular, the "minimally-ablative CO_2 fractional laser", thus creating a sort of "cross-roads" between the effectiveness of ablative and the safety of non-ablative sources. Fractional resurfacing is a method of treating thermal injury in numerous microscopic areas with controlled width, depth, and density, surrounded by a reservoir of protected epidermal and dermal tissue, thus enabling rapid repair of laser-induced thermal injury. To clinically, histologically and ultrastructurally evaluate the safety and efficacy of the fractional CO_2 laser in the treatment of photo-damaged skin, with special attention to a specific clinical parameter of this kind of laser: the Stacking Mode. The aim of our work was to compare how different CO_2 laser powers, by modulating the secretory pathway of cytokines, are able to influence the wound healing process, and how these powers and other important parameters such as the Stacking mode, are associated with different clinical results. Histological and ultrastructural changes were also assessed. Our study focused overall on the identification of collagen morphological alterations using Multiphoton microscopy, a new method for non-invasive evaluation of the molecular structure of the dermis

We report the results of the first morphological alteration of collagen after fractional CO_2 laser treatment. Our study shows how Fractional CO_2 laser is a safe and promising approach for the treatment of visible signs of ageing, and how multiphoton microscopy can be considered a new method of non-invasive evaluation of the molecular structure of the dermis, thus avoiding skin biopsy. The positive results suggest that working in this field is a complex issue that requires a great deal of experience and knowledge of laser-tissue interactions.

References

1) Campolmi P., Bonan P.,Cannarozzo G. I laser e le sorgenti luminose in dermatologia. Masson, 2003 **2)** Cannarozzo G., Campolmi P., Bonan P., Buggiani G., Dindelli M., Zingoni T., Lotti T. CO_2 laser . Applicazioni in dermatologia. Hi.tech dermo 5/2008, pag 31 – 42 **3)** F. Prignano, P. Campolmi, P. Bonan, F. Ricceri, G. Cannarozzo, M. Troiano & T. Lotti. Fractional CO_2 laser: a novel therapeutic device upon photobiomodulation of tissue remodeling and cytokine pathway of tissue repair. Dermatologic Therapy 2009, Vol. 22, Issue s1, Pages S8 – S11 **4)** Riccardo Cicchi, Dimitrios Kapsokalyvas, Vincenzo De Giorgi, Vincenza Maio, Annelies Van Wiechen, Daniela Massi, Torello Lotti, Francesco S. Pavone. Scoring of collagen organization in healthy and diseased human dermis by multiphoton microscopy. Journal of Biophotonics 2010, Vol 3, Issue 1 - 2, pag 34 – 43 **5)** R. Cicchi, S. Sestini, V. De Giorgi, D. Massi, T. Lotti, F. S. Pavone. Nonlinear laser imaging of skin lesions. Journal of Biophotonics 2008, Vol 1, Issue 1, pag 62 – 73


SFP
b


LUX
VITA
EST


cnrs
dépasser les frontières


MAIRIE DE PARIS


L'ORÉAL
PARIS


GALDERMA


ESP