ESP Photobiology School

June 16 – 21, 2014

Brixen/Bressanone

Programme

Abstracts

European Society for Photobiology

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Lesley Rhodes (UK, ESP President)
Giorgia Miolo (IT, Local Organizer)
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Stefan Anderson-Engels (SE), Roberto Bassi (IT), Kristian Berg (NO), Giovanni Checcucci (IT), Olimpia Coppellotti (IT), Giulio Jori (IT), Herwig Kostron (AT), Miguel Miranda (ES), Tomas Morosinotto (IT), Carlo Musio (IT), Santi Nonell (ES), Jacques Piette (BE), Lesley Rhodes (UK), David Russell (UK), Evelyne Sage (FR), Rolf-Markus Szeimies (DE), Franz Trautinger (AT), Massimo Trotta (IT), Rex M.Tyrrell (UK), Georges Wagnieres (CH)

Important locations in Brixen/Bressanone

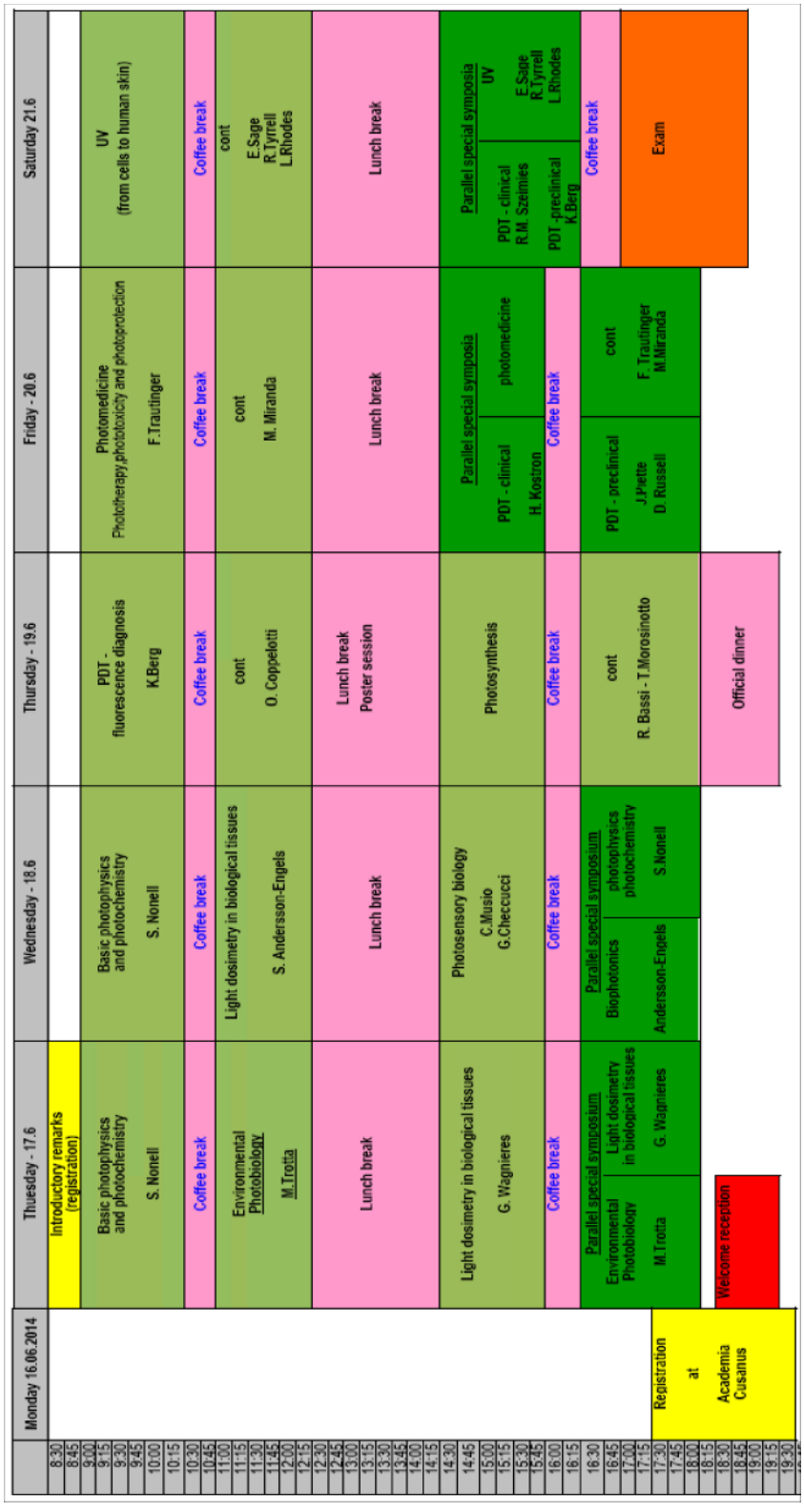
School venue:
Casa della Gioventù, Università di Padova,
Address:
via Rio Bianco 6.
39042 Bressanone BZ, Italy

Accomodation:
Academia Cusanus
Address:
Via del Seminario 2, 39042 Bressanone BZ, Italia

Sponsor

European Society for Photobiology

ESP Photobiology School - Time Schedule



ESP PHOTOBIOLOGY SCHOOL
SCIENTIFIC PROGRAM

Monday June 16

Time: **17:30-19:30:**

Registration - at Academia Cusanus

Tuesday June 17

Time: **8:30-9:00: Welcome and Introductory remarks**

Time: **9:00-10:30**

Topic, general: **Basic photophysics and photochemistry**
Lecturer:
Santi Nonell, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain.

Time: **10:30-11:00: Coffee break**

Time: **11:00-12:30**

Topic, general: **Environmental photobiology**
Lecturer:
Massimo Trotta, Institute for Chemical-Physical Processes, Italian National Research Council (CNR), Bari, Italy

12:30 – 14:30 Lunch

Time: **14:30-16:00**

Topic, general: **Light dosimetry in biological tissues**
Lecturer:
Georges Wagnieres, Institute of Chemical Sciences and Engineering, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland.

Time: **16:00-16:30: Coffee break**

Parallel sessions

Time: **16:30-18:00**

I. ***Topic, special:*** **Environmental photobiology**
Lecturer:
Massimo Trotta, Institute for Chemical-Physical Processes, Italian National Research Council (CNR), Bari, Italy

II. ***Topic, special:*** **Light dosimetry in biological tissues**
Lecturer:
Georges Wagnieres, Institute of Chemical Sciences and Engineering, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland.

Time: **19:00-20:00:**

Welcome reception - at the School venue

Wednesday June 18

Time: **9:00-10:30**

Topic, general: **Basic photophysics and photochemistry**
Lecturer:
Santi Nonell, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain.

Time: **10:30-11:00: Coffee break**

Time: **11:00-12:30**

Topic, general: **Light dosimetry in biological tissues**
Lecturer:
Stefan Andersson-Engels, Department of Physics, Lund University, Lund, Sweden.

12:30 – 14:30 Lunch

Time: **14:30-16:00**

Topic, general: **Photosensory biology**
Lecturers:
Carlo Musio, Consiglio Nazionale delle Ricerche - Istituto di Biofisica Fondazione Bruno Kessler, Trento, Italy
Giovanni Checcucci, Istituto di Biofisica, Consiglio Nazionale delle Ricerche, Pisa Italy

Time: **16:00-16:30: Coffee break**

Parallel sessions

Time: **16:30-18:00**

I. ***Topic, special:*** **Light dosimetry in biological tissues**
Lecturer:
Stefan Andersson-Engels, Department of Physics, Lund University, Lund, Sweden.

II. ***Topic, special:*** **Photophysics and photochemistry: Measurement, simulation, and analysis of spectroscopic data**
Lecturer:
Santi Nonell, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain.

Thursday June 19

Time: **9:00-12:30**

Topic, general: **Photodynamic medicine: On the way towards bedside**

Time: **9:00-10:30:** **Photodynamic medicine: From basics to practice**
Lecturer:
Kristian Berg, The Norwegian Radium Hospital, Department of Radiation Biology, Oslo, Norway

Time: **10:30-11:00:** **Coffee break**

Time: **11:00-12:30:** **Antimicrobial photodynamic therapy (PDT)**
Lecturer:
Olimpia Coppellotti, Department of Biology, University of Padova, Padova, Italy.

12:30 – 14:30 Lunch and Poster session

Time: **14:30-18:00**

Topic, general: **Photosynthesis**

Time: **14:30-16:00:** **Photosynthesis**
Lecturer:
Tomas Morosinotto, Department of Biology, University of Padova, Italy

Time: **16:00-16:30:** **Coffee break**

Time: **16:30-18:00:** **Photosynthesis**
Lecturer:
Roberto Bassi, Department of Biotechnology, University of Verona, Italy

Afternoon: Official dinner

Friday June 20

Time: **9:00-12:30**

Topic, general: **Photomedicine (Phototherapy, phototoxicity and photoprotection)**

Time: **9:00-10:30** **Basic photodermatology**
Lecturer:
Franz Trautinger, Karl Landsteiner Institute for Dermatological Research, St. Poelten, Austria

Time: **10:30-11:00** **Coffee break**

Time: **11:00-12:30** **Photoreactivity and phototoxicity of drugs**
Lecturer:
Miguel Miranda, Universidad Politecnica de Valencia, Departamento de Quimica, Valencia, Spain

12:30 – 14:30 Lunch

Parallel sessions

I. ***Time:*** **14:30-16:00** ***Topic, special:*** **PDT – clinical**
PDD/PDT in Neurosurgery- clinical applications (Kostron)
Lecturer:
Herwig Kostron, Department of Neurosurgery, Medical University of Innsbruck, Innsbruck, Austria

Time: **16:00-16:30** **Coffee break**

I. ***Time:*** **16:30-18:00** ***Topic, special: PDT – preclinical***
Mechanisms by which tumor cell die or survive after photodynamic therapy
Lecturer:
Jacques Piette, Laboratory of Virology & Immunology, University of Liege, Liege, Belgium
Nano-systems for photodynamic therapy (Russell)
Lecturer:
David Russell, School of Chemistry, University of East Anglia, Norwich, Great Britain

II. ***Time:*** **14:30-18:00** ***Topic, special:*** **Photomedicine (Phototherapy, phototoxicity and photoprotection)**

Time: **14:30-16:00** ***Topic, special:*** **Phototherapy: Specific treatment modalities**
Lecturer:
Franz Trautinger, Karl Landsteiner Institute for Dermatological Research, St. Poelten, Austria
Time: **16:00-16:30** **Coffee break**

Time: **16:30-18:00** ***Topic, special:*** **Photooxidative reactions of drugs with biomolecules**
Lecturer:
Miguel Miranda, Universidad Politecnica de Valencia, Departamento de Quimica, Valencia, Spain

Saturday June 21

Time: **9:00-12:30** ***Topic, general:*** **UV (from cells to skin tissue)**

Solar UV-induced DNA damage, repair, mutagenesis and carcinogenesis
Lecturer:
Evelyne Sage, Institut Curie – Recherche, Centre Universitaire, Orsay – France

Solar UV generation and biological significance of reactive oxygen species
Lecturer:
Rex Tyrrell, Department of Pharmacy & Pharmacology,University of Bath, United Kingdom

Solar UVR-induced vitamin D synthesis
Lecturer:
Lesley Rhodes, Photobiology Unit, Dermatological Sciences, Salford Royal Foundation Hospital, University of Manchester, Manchester - United Kingdom.

12:30 – 14:30 Lunch

Parallel sessions

Time: **14:30-16:30**

I. ***Topic, special: PDT – clinical***
Photodynamic Therapy in Dermatology – From experimental status to routine therapy
Lecturer:
Rolf-Markus Szeimies, Department of Dermatology and Allergology, Vest Clinic, Recklinghausen, Germany

I. ***Topic, special: PDT – preclinical***
Photochemical internalization (PCI) – from photodynamic targeting of lysosomes to clinical utilization of PCI
Lecturer:
Kristian Berg, The Norwegian Radium Hospital, Department of Radiation Biology, Oslo, Norway

II. ***Topic, special:*** **UV (from cells to skin tissue)**
A role for UVA on skin cancer
Lecturer:
Evelyne Sage, Institut Curie – Recherche, Centre Universitaire, Orsay – France

Endogenous and exogenous protection against UV generated oxidative stress
Lecturer:
Rex Tyrrell, Department of Pharmacy & Pharmacology,University of Bath, United Kingdom

Balancing the benefits and risks of solar UVR exposure
Lecturer:
Lesley Rhodes, Photobiology Unit, Dermatological Sciences, Salford Royal Foundation Hospital, University of Manchester, Manchester - United Kingdom.

Saturday June 21

Time: **17:00-20:00**

EXAM

Lecture Abstracts

Preferential reading has been highlighted in bold

Tuesday morning, *topic, basic:*
Photophysics and Photochemistry (Nonell)

Light
Nature and properties of light. Light sources in photobiology. Light conditioning and measuring

Interaction of light with biomolecules
Outcomes of the interaction of light with matter. Light absorption: creating excited states. The properties of excited states. Absorption spectra and action spectra

Photophysics
The fate of excited states: radiative and non-radiative unimolecular decay processes. Rate constants, quantum yields, and lifetimes. A closer look to fluorescence. Emission techniques: steady-state and time-resolved. Fluorescence microscopy. Fluorescent proteins. Photothermal techniques

Photochemistry
General aspects. Classification of photochemical reactions. Selected examples in Photobiology

Tuesday morning, *topic, basic;*
Environmental photobiology (Trotta)

1. DEFINITION OF PHOTOBIOLOGY (LIFE UNDER THE SUN)
2. BRIEF INTRODUCTION IN A PLAUSIBLE BREAKDOWN OF TOPICS INCLUDED UNDER THE TERM PHOTOBIOLOGY
3. FOCUS ON ENVIRONMENTAL PHOTOBIOLOGY
   A. ROLE OF TERRESTRIAL PLANTS (WITHOUT ENTERING IN DETAILS ON PHOTOSYNTHESIS WHICH HAS ITS OWN LECTURE ON THURSDAY)
   B. AQUATIC SYSTEMS
   C. LIGHT AND PLANET ENERGY
   D. CLIMATE ISSUES INCLUDING OZONE CHEMISTRY
   E. ECOSYSTEMS DOMINATED BY LIGHT
4. SUMMARY

Tuesday after lunch and Wednesday, *basic and special symposia*:
Light dosimetry in biological tissues (Wagnieres and Andersson-Engels)

Course description:
The main objective of this course is to convey a broad introduction to the principles governing the propagation of light as well as its interactions with biological tissues, including plants. Consequently, the fundamental optical parameters and the photophysical processes (absorption, scattering, fluorescence) involved in these interactions will be defined and described. Following a very brief presentation of the scientific, medical and financial advantages of biomedical photonics, different approach to model the propagation of light will be presented. In addition, the measurement and calculation of the light dose in biological tissues will be described. This will require addressing fundamental concepts in the fields of radiometry and photometry. The instrumentation and techniques used to illuminate or to measure the light in biological tissues will be described. Finally, selected illustrative applications will be presented.
This course will provide an excellent background to enable the communication and interaction between the students and industrial/laboratory specialists, as well as with medical or clinical partners, thanks to a good understanding of the vocabulary, principles and instruments used in these fields.

Chapter 1 in the PHD thesis from Tomas Svensson and Chapter 1&2 from the PhD thesis of Johan Axelsson. The theses can be found on our homepage:
http://www.atomic.physics.lu.se/biophotonics/publications/phd_theses/

http://www.optics.arizona.edu/Palmer/rpfaq/rpfaq.htm

A.J. Welch & M.J.C. van Gemert. Optical-Thermal Response of Laser Irradiated Tissue **(2nd Ed., Springer, 2011)**

V. Tuchin, "Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis", volume PM166, SPIE Press, 2nd Edition, 2007.

J. Sandell and T. Zhu, "A review of in-vivo optical properties of human tissues and its impact on PDT", J Biophotonics", 4(11-12), pp 773–787, 2011.

Tuesday after lunch, *topic special:*
Environmental photobiology (Trotta)

Wednesday morning, *Topic, general:*
Basic photophysics and photochemistry (Nonell)

Energy transfer
Bimolecular decay processes. Quenching and the Stern-Volmer relationship. Energy transfer: concepts and mechanisms. FRET and its applications. Singlet oxygen: production, characterisation and detection.

Electron transfer
Thermodynamic and kinetic aspects. Charge-transfer derived Reactive Oxygen Species (ROS). Time-resolved absorption techniques: Detection of radicals and radical ions.

Bibliography

G. Cox. *Optical Imaging techniques in Cell Biology*, CRC, Boca Raton, 2007

Peter Klán and Jakob Wirz, *Photochemistry of Organic Compounds*, John Wiley & Sons 2009

Brian Wardle, Principles and Applications of Photochemistry, John Wiley & Sons 2009
N. J. Turro, V. Ramamurthy, J. C. Scaiano, *Modern Molecular Photochemistry of Organic Molecules*, University Science Books; 2009.

Lars Olof Björn (Ed.), Photobiology, *The Science of Life and Light*, Springer, 2008

Handbook of Photochemistry, Third Edition (Hardcover)

Marco Montalti, Alberto Credi, Luca Prodi, M. Teresa Gandolfi, Handbook of photochemistry 3rd Edition, Marcel Dekker, 2006

Photobiology Sciences Online, http://www.photobiology.info/

Wednesday after lunch, *topic, basic:*
Photosensory biology (Musio and Checcucci)

Non-Visual Photoreception and Image-Forming Vision in Metazoa (Carlo Musio)
(carlo.musio@cnr.it

Photoreception is the first key step in seeing (*i.e.* the perception of color, shape and motion). It takes place in photoreceptor cells capable to sense directly ambient light. Visual pigments are integral membrane proteins of photoreceptor cells, which absorb photon energy and finally convert it into an electrical signal toward the central nervous system. Photoreception is phylogenetically one of the oldest sensory systems due to the amazing ubiquity, in all animal *phyla*, of morphological, functional and molecular systems (from simple invertebrate light-sensitive cells to more complex vertebrate eyes) that respond to environmental luminous stimuli. Basically, although in a frame of different structure-function relationships, in vertebrate and invertebrate visual cells photoreception starts with the photoisomerization of the retinal cromophore of the photopigment, usually an opsin-based pigment. This process triggers the binding of the opsin with a G-protein which leads an enzymatic visual cascade culminating in the production of a second messenger, which gates light-sensitive ion channels in order to modulate and shape the electric signal toward the nervous system. In addition to conventional eyed-structures, vertebrates and invertebrates have supplementary non-visual photoreceptor (NVP) systems for non-image forming function. Photic information mediated by NVP

integrates visual activity and is involved in temporal (time-of-day) and behavioral physiology of the animal (*e.g.*, photoperiodism, photoentrainment of circadian rhythms). NVP photoreceptors, formerly named extraretinal or extraocular, are currently termed as non-visual (non-image-forming) photosensitive cells in invertebrates and non-rod non-cone photoreceptors in vertebrates (after the discovery of photosensitive retinal ganglion cells ipRGCs). NVP cells are mainly located within nervous system districts and share with retinal photoreceptors common evolutionary origin and light-sensing modalities: above all the same superfamily of opsin-based photopigment although with variations in structural motifs and related phototransductive elements. The searching for novel opsins (e.g., melanopsin) supplying non-image-forming photoreception is a new challenging field in photosensory biology and in vision research. Surprisingly, these pigments have been identified in cells beyond the retinal photoreceptors in several vertebrates and in extraretinal tissues of invertebrates. Recently, genetically-engineered visual (rhodopsin) non-visual (melanopsin) and microbial opsins (e.g., channelrhodopsins), have been proved as optogenetic tools to control physiology and behavior in cells and organisms. This lecture will survey similarities and differences among visual and non-visual systems in both vertebrates and invertebrates, focusing on the role of opsin-based photopigments and how almost similar structural outlines and biophysical mechanisms could serve different physiological tasks. Recent insights on optogenetic control exerted by opsins will be provided too.

SELECTED READINGS

Crouch R & Y Shichida, eds (2010) Photosensitive retinal pigments. *Photochem. Photobiol. Sci*, 9(11):1409-1524 [themed issue with 13 multi-authored review articles].

Han X (2012) In vivo application of optogenetics for neural circuit analysis. *ACS Chem Neurosci*. 3: 577-584

Musio C & S Santillo (2012) Nonvisual photosensitivity and circadian vision. In: *CRC Handb. Organic Photochem. Photobiol.*, 3rd ed (Griesbeck A, Oelgemöller M, Ghetti F, eds), CRC Press, pp. 1195–1210.

Santillo S, P Orlando, L De Petrocellis, L Cristino, V Guglielmotti & C Musio (2006) Evolving visual pigments: hints from the opsin-based proteins in a phylogenetically old eyeless invertebrate. *BioSystems* 86:3-17.

Sexton T, E Buhr, RN Van Gelder (2012) Melanopsin and mechanisms of non-visual ocular photoreception. *J Biol Chem.* 287:1649-56.

Terakita A (2005) The opsins. *Genome Biol.* 6:213 http://genomebiology.com/2005/6/3/213

Yau KW & RC Hardie (2009) Phototransduction motifs and variations. *Cell* 139:246–264.

PHOTOSENSORY BIOLOGY IN ANEURAL ORGANISMS (Giovanni Checcucci)

(giovanni.checcucci@pi.ibf.cnr.it)

Gathering information on the surrounding environment is of vital importance for any living organism. Therefore also aneural organisms (prokaryotic and eukaryotic unicellular microorganisms, fungi, plants) have developed ways of perceiving and reacting to the luminous stimulus. The reaction in microorganisms is usually a change in movement pattern, that mainly evolved because of the necessity of optimizing photochemical energy production or avoiding photoinduced damage, but that may also be correlated to the presence of preys (e.g. photosynthetic algae), predators or favour the possibility of reproducing. In sessile organisms responses to light are even more important and allow the individual to react to changes in light quality (tip elongation), quantity (leaves orientation, chloroplasts movement), duration (flowering) and direction (phototropism).

This lecture will mainly deal with the basic principles of photoperception and photoreaction, analysing the structure and function of known photoreceptors, the molecular pathways that transduce the light stimulus to a chemical signal, and how this affects and modulates the effector.

REFERENCES

www.photobiology.info - Web site entirely devoted to photobiology. Of particular interest for photosensory biology of aneural organisms are the modules, "Photomorphogenesis" and "Photomovement".

CRC Handbook of Organic Photochemistry and Photobiology, Third Edition (Griesbeck A., Oelgemöller M., Ghetti F., eds), CRC Press. - Several chapters of the photobiology section deal with photosensory biology.

Möglich A., Yang X., Ayers R.A., Moffat K. (2010) Structure and function of plant photoreceptors. Annual Review of Plant Biology, 61: 1-27

Sgarbossa A., Checcucci G., Lenci F. (2002) Photoreception and photomovements of microorganisms. Photochemical and Photobiological Sciences, 1: 459-467.

Wednesday after lunch, *special topic*:
Photophysics and photochemistry: Measurement, simulation, and analysis of spectroscopic data (Nonell).

Solar irradiance data and the UV Index. Absorption spectra of the aminoacids and nucleobases. Absorption spectra of UV photoprotectors. Absorption spectra of endogenous photosensitisers. Assessing donor-acceptor pairs for Förster resonance energy transfer. Analysis of fluorescence decay data. The singlet oxygen simulator.

Please make sure you have the PhotochemCAD software installed on your computer at the beginning of the course (available at www.photochemCAD.com)

Bibliography

J. M. Dixon, M. Taniguchi, J. S. Lindsey, PhotochemCAD 2. A Refined Program with Accompanying Spectral Databases for Photochemical Calculations," Photochem. Photobiol. 2005, 81, 212-213.

<u>Thursday morning</u>, *topic, basic:*
Photodynamic medicine: From basics to practice (Berg and Coppellotti)

Basic PDT and use in cancer detection and treatment (Kristian Berg)

Photodynamic therapy (PDT) is a clinically approved, minimally invasive therapeutic procedure that can exert a selective cytotoxic activity toward malignant cells. The procedure involves administration of a photosensitizing agent followed by irradiation at a wavelength corresponding to an absorbance band of the sensitizer. In the presence of oxygen, a series of events lead to direct tumor cell death, damage to the microvasculature, and induction of a local inflammatory reaction. This lecture will present the basic mechanisms involved in PDT and fluorescence diagnosis (PD) such as formation of reactive oxygen species, cellular uptake mechanisms and intracellular localization of the photosensitizers, cellular formation of photosensitizers, pharmacokinetics of photosensitizers, the impact of light penetration and light sources on therapeutic effects and some information on the clinical use of PDT and PD.

Berg et al. (2005) Porphyrin-related photosensitizers for cancer imaging and therapeutic applications. *J Microscopy* 218(Pt 2):133-47.
Agostinis et al. (2011) Photodynamic Therapy Of Cancer: An Update CA: A Cancer Journal for Clinicians. 61(4):250-81.
http://www.photobiology.info/Kessel.html

Antimicrobial photodynamic therapy (PDT) (Olimpia Coppellotti)

Photodynamic therapy (PDT) is emerging as a promising and efficient alternative treatment for microbial infections, a problem which is presently exasperated by the increasingly widespread diffusion of antibiotic-resistant microbial strains. Studies on the relationship between the chemical structure of photosensitizing agents and their phototoxicity against microbial pathogens led to identification of a selected number of compounds with optimal cytocidal effects. In particular, the use of red light-absorbing photosensitizers as photodynamic antimicrobial agents is characterized by various favourable features, including: (a) the broad spectrum of antimicrobial action of selected phenothiazines, porphyrins, and phthalocyanines, which promote the photosensitized inactivation of Gram(+) and Gram(-) bacteria, fungi, mycoplasmas, and parasitic protozoa in both vegetative and cystic stages, by using one phototherapeutic protocol and mild irradiation conditions; (b) porphyrins/phthalocyanines display no appreciable toxicity in the dark of photochemically active doses; (c) microbial cell death is primarily a consequence of membrane photodamage through a typically multi-target process, which minimizes the risk of both the onset of mutagenic processes and the selection of photoresistant cells; (d) such photosensitizers act with essentially identical efficiency against both wild and antibiotic resistant strains, whereas no selection of photoresistant microbial pathogens has been observed; (e) a combination between antibiotic-based and photodynamic therapy is possible. The primary interaction of the photosensitizer takes place with the outer wall that surrounds most microbial cells. This wall is characterized by broadly different levels of complexity and three-dimensional architecture, as well as of permeability to external molecules. A typical example of phthalocyanine-sensitized photoinactivation of methicillin-resistant *Staphylococcus aureus* (MRSA) is provided. MRSA has become a predominant infective agent even in nosocomial environments due to its ability to develop high levels of resistance to several classes of antibiotics through different pathways, including mutation, conjugation, transduction or transformation. PDT appears to represent an efficacious alternative modality for the

treatment of localized microbial infections through the *in situ* application of the photosensitizer followed by irradiation of the photosensitizer-loaded infected area. Proposed clinical fields of interest of antimicrobial PDT include the treatment of chronic ulcers, infected burns, acne vulgaris, and a variety of oral infections, such as oral candidiasis and periodontitis.

Selected readings

Jori G., Fabris C., Soncin M., Ferro S., Coppellotti O., Dei D., Fantetti L., Chiti G., Roncucci G. (2006). Photodynamic therapy in the treatment of microbial infections: Basic principles and perspective applications. *Lasers Surgery and Medicine*, **38**: 468-481.

Jori G., Camerin M., Soncin M., Guidolin L., Coppellotti O. (2011). Antimiocrobial photodynamic therapy: basic principles. In "Photodynamic inactivation of microbial pathogens: medical and environmental applications" (Michael R. Hamblin & Giulio Jori Editors). Comprehensive Series in Photochemistry and Photobiology, Volume 11, 1- 18. RSC Publishing, Cambridge, UK.

Coppellotti O., Fabris C., Soncin M., Magaraggia M., Camerin M., Jori G., Guidolin L. (2012). Porphyrin photosensitised processes in the prevention and treatment of water- and vector-borne diseases. *Current Medicinal Chemistry*, **19**: 808-819.

Thursday after lunch, *topic, basic:* Photosynthesis (Bassi and Morosinotto)

Photosynthesis (Morosinotto)

This part of the lecture will review of the light dependent reactions of oxygenic photosynthesis and how light is absorbed by photosynthetic complexes and converted into photochemical energy. It will also describe protein composition of photosystems, the molecular machineries catalysing the primary steps of light conversion into the chemical bond energy of organic compounds. In higher plants, algae and cyanobacteria, these steps are operated by two pigment-protein supercomplexes localised in the thylakoids membranes, called Photosystem I (PSI) and II (PSII). Their activity leads to the transport of electrons from the water to a final acceptor with higher potential (NADP+) as well as to a asymmetric protons and charge distribution, which is the motor force for ATP synthesis. The peculiarity of Photosystems with respect to the other chloroplast complexes is their binding of massive amounts of pigmented compounds, chlorophylls and carotenoids.
Photosystems are composed by two moieties: attention will be first dedicated to the core complexes, which are responsible of conversion of light into chemical energy. Later we will describe antenna systems, which are composed by pigment binding proteins responsible of increasing light harvesting capacity. While core complexes are conserved, antenna systems diverged during evolution and differences between different organisms capable of oxygenic photosynthesis (cyanobacteria, algae and plants) will be described. Particular attention will also be dedicated to how proteins of the photosynthetic apparatus are able to modulate pigments biophysical properties.

Photosynthesis (Bassi)

This part of the lecture will instead focus on the regulation of photosynthesis light reactions. In a natural environment, light for photosynthetic organisms represents not only an energy supply but also a source of reactive oxygen species, when light absorbed is in excess. Plants are particularly exposed to oxidative stress because they live in a land environment where illumination is generally stronger and oxygen diffusion is faster with respect to water. To avoid cellular damages different

photoprotection mechanisms evolved, allowing plants survival in these conditions. Differences with other photosynthetic organisms living in water will also be reviewed. In particular, we will describe recent findings on the faster among photoprotection mechanisms called NPQ (Non Photochemical Quenching). This is particularly interesting because it allows modulation of light harvesting efficiency within a few seconds without requiring protein synthesis or post-translational modifications. This is a valuable example on how protein conformational changes are able to drastically modulate quantum yield of pigments they are binding.
The relevance of the clarification of regulation of photosynthesis in the exploitation of photosynthetic organisms for biofuels production will be also discussed.

Bibliography for Photosynthesis

Chapter 12 on Photosynthesis from “Biochemistry and Molecular Biology of Plants” by Bob B. Buchanan, Wilhelm Gruissem, and Russell L. Jones. ASPB publications

Nelson N. Photosystems and global effects of oxygenic photosynthesis. Biochim Biophys Acta. 2011 Aug;1807(8):856-63. doi: 10.1016/j.bbabio.2010.10.011. Epub 2010 Oct 16.

Eberhard S, Finazzi G, Wollman FA. The dynamics of photosynthesis. Annu Rev Genet. 2008;42:463-515.

Silvia de Bianchi, Matteo Ballottari, Luca Dall’Osto and Roberto Bassi. Regulation of plant light harvesting by thermal dissipation of excess energy. Biochem Soc. Trans. 2010

Li Z, Wakao S, Fischer BB, Niyogi KK. Sensing and responding to excess light. Annu Rev Plant Biol. 2009;60:239-60. Review.

Niyogi KK, Truong TB. Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. Curr Opin Plant Biol. 2013 Jun;16(3):307-14. doi: 10.1016/j.pbi.2013.03.011

<u>Friday morning</u>, *topic, basic:*
Photomedicine (Trautinger and Miranda)

Basic photodermatology (Trautinger)

Abstract: Photodermatology deals with the clinical consequences of the interaction of ultraviolet radiation with the various molecular and cellular components of human skin. These responses can be either physiologic (e.g. Vitamin D production, tanning) or lead to adverse reactions and disease (e.g. sunburn, polymorphic light reaction, and other photosensitivity diseases). Furthermore, sunlight and ultraviolet radiation from specific artificial sources (UVB, narrow-band UVB, UVA1) alone or in combination with photosensitizing drugs (psoralen-photochemotherapy) can be therapeutically employed for the treatment of a wide range of skin and other diseases. Finally, photodermatology also includes photoprotection through avoidance, clothing, and sunscreens with the main aim to prevent photoaging and photocarcinogenesis.
The lecture will provide basic knowledge about these major areas in photodermatology.

Readings:
Effects of ultraviolet radiation, visible light, and infrared radiation on erythema and pigmentation: a review
Lindsay R. Sklar, Fahad Almutawa, Henry W. Lim and Iltefat Hamzavi, Photochem. Photobiol. Sci., 2013, 12, 54-64
DOI: 10.1039/C2PP25152C

Photoreactivity and phototoxicity of drugs (Miranda)

- Photoreactivity of drugs under sunlight: photostability and its implications.
- Drugs as photosensitizers: desired and undesired effects.
- Major types of photosensitizing drugs.
- Predicting and understanding the phototoxicity of drugs: screening tests and mechanistic assays.
- Photophysics of drugs. Fluorescence measurements and laser flash photolysis studies.
- Photochemistry of drugs. Identification of photoproducts and elucidation of the involved photochemical mechanisms.
- Interaction of drug excited states with biomolecules.
- Photosensitization by drug metabolites and photoproducts.
- Photochemistry of sunscreens: photoprotection, photostability and photosensitization.

<u>Friday after lunch</u>, *topic special*:
PDT – clinical (Kostron)

PDD/PDT in Neurosurgery- clinical applications (Kostron)

Scope:
After the lecture the attendies should be have knowledge about clinical photodynamic application in Neurosurgery including

- pathological entities
- neuroimaging
- indication
- what sensitizer
- PDD and PDT and combination of both
- which light dose regime
- post treatment care

Outline:
With current treatment methods the prognosis for patients with aggressive brain tumors is dismal with a median survival of 15 months. Treatment failure is usually due to local recurrence of tumor. Intra-operative photodynamic detection (PDD) of tumor tissue and post-surgical photodynamic therapy (PDT) of the resection cavity may be of benefit.
Photodynamic techniques such as photodynamic diagnosis (PDD) , fluorescence guided tumor resection (FGR) and photodynamic therapy (PDT) are undergoing intensive clinical investigations as adjunctive treatment for malignant brain tumours.

At the beginning a short outline of the neuropathological entities will be presented as well as diagnostic tools such as MRI, CT and PET.
This will be followed by basics of photomedicine in neurooncology which differes from the general oncological and non-oncological situation.
A historical review upon the development of PDT will be given.

In the following the indications for PDD and PDT in braintumors will be discussed.
Due to special anatomical situation of the brain specific features and requirements of the sensitizers have to be fulfilled.
Light and lasertechnologies for fluorescenguided resection and interstitial light delivery will be presented. Practical examples will be presented.
Furthermore various light dose regimes for high and metronomic light delivery are demonstrated and discussed.
Real clinical cases for practical demonstration will be shown and discussed in detail.

This session will be concluded with an overview on the current clinical data and trials as well as on future of photodynamic application in neurosurgery especially in the light of current standard treatment options .

Li M, Deng H, Peng H, Wang Q Functional nanoparticles in targeting glioma diagnosis and therapies.
J Nanosci Nanotechnol. 2014 Jan;14(1):415-32.

Photodynamic therapy of malignant brain tumours: a complementary approach to conventional therapies.
Bechet D, Mordon SR, Guillemin F, Barberi-Heyob MA.
Cancer Treat Rev. 2014 Mar;40(2):229-41

Kostron H: Photodynamic diagnosis and therapy for brain malignancies; from bench to clinical application. In "Photodynamic Therapy- from theory to application" ed M H Abdel-Kader, Springer 165-184.

Muragaki Y, Akimoto J, Maruyama T, Iseki H, Ikuta S, Nitta M, Maebayashi K, Saito T, Okada Y, Kaneko S, Matsumura A, Kuroiwa T, Karasawa K, Nakazato Y, Kayama T.
Phase II clinical study on intraoperative photodynamic therapy with talaporfin sodium and semiconductor laser in patients with malignant brain tumors.
J Neurosurg. 2013 Oct;119(4):845-52.

Protoporphyrin IX fluorescence and photobleaching during interstitial photodynamic therapy of malignant gliomas for early treatment prognosis.
Johansson A, Faber F, Kniebühler G, Stepp H, Sroka R, Egensperger R, Beyer W, Kreth FW.
Lasers Surg Med. 2013 Apr;45(4):225-34. doi: 10.1002/lsm.22126. Epub 2013 Mar 26

Gefitinib enhances the efficacy of photodynamic therapy using 5-aminolevulinic acid in malignant brain tumor cells.
Sun W, Kajimoto Y, Inoue H, Miyatake S, Ishikawa T, Kuroiwa T.
Photodiagnosis Photodyn Ther. 2013 Feb;10(1):42-50.

Ritz R, Daniels R, Noell S, Feigl GC, Schmidt V, Bornemann A, Ramina K, Mayer D, Dietz K, Strauss WS, Tatagiba M.
Hypericin for visualization of high grade gliomas: first clinical experience.
Eur J Surg Oncol. 2012 Apr;38(4):352-60

Lyons M, Phang I, Eljamel S.
The effects of PDT in primary malignant brain tumours could be improved by intraoperative radiotherapy.
Photodiagnosis Photodyn Ther. 2012 Mar;9(1):40-5

<u>Friday after lunch</u>, *topic special*:
PDT – preclinical (Piette and Russell)

Mechanisms by which tumor cell die or survive after photodynamic therapy (Jacques Piette)
(jpiette@ulg.ac.be)
Photodynamic therapy (PDT) is an important modality of tumor treatment based on the use of a photosensitizer, oxygen and light. Reactive oxygen species, and particularly singlet oxygen, have been demonstrated to be important mediators of the cellular response to PDT. In this lesson we will focus on the mechanisms initiated by PDT leading to tumor cell death and survival.
Direct cell death is an important mediator of tumor eradication by PDT. Several cell death modalities will be reviewed such as apoptosis and the various forms of regulated necrosis such as necroptosis, ferroptosis and oxytosis. Immunological cell death which is an important indirect cell death mechanism will also be reviewed together with its impact on the possible vaccination against tumor.
Since tumor cells have been shown to be capable of surviving and regrowing after the cellular stress induced by PDT, these molecular mechanisms contributing to cancer cell survival will also be reviewed. We will focus first on autophagy that has been primarily described as a pro-survival mechanism but can also be as seen as a double-edge sword also involved in cell consumption and apoptosis.
Finally cancer cells that survived to PDT modify their genetic programs by switching on and off various transcription factor controlling the expression of many genes involved in cell survival. Two signal transduction mechanisms leading transcription factor activation will be reviewed: the one leading to NF-kB activation and the other leading to Nrf-2 activation.
This lesson should bring to students an updated view on how tumor cells may die or survive to PDT and how these mechanisms could be influenced in order to increase PDT efficiency.

Paper 1: Self-consuption: the interplay of autophagy and apoptosis by G. Marino et al (2014) Nature Rev Mol Cell Biol 15, 81-94
Paper 2: Regulated necrosi: the expanding network of non-apoptotic cell death pathways by Vanden Berghe et al (2014) Nature Rev Mol Cell Biol 15, 135-147

Nano-systems for photodynamic therapy (Russell)
(d.russell@uea.ac.uk)
One of the areas where nanotechnology is likely to have a profound influence in the development of novel therapies is within medicine. Nanomedicine has now become a discipline where numerous groups Worldwide have focused research activity. This presentation will provide a review of some of the activities within nanomedicine that have been developed for photodynamic therapy (PDT). Nano-systems such quantum dots, silica and metallic nanoparticles and upconverting nanoparticles will all be considered. The development of multifunctional nano-systems where a targeting molecule, such as an antibody, has been added with the photoactive photosensitiser will be discussed. An assessment will be made of the importance of nanomedicines for the efficient delivery of photosensitiser agents and their therapeutic efficacy.

Suggested reading:

1. Nanoparticles as vehicles for delivery of photodynamic therapy agents, D. Bechet, P. Couleaud, C. Frochot, M-L Viriot, F. Guillemin and M. Barberi-Heyob, *Trends in Biotechnology*, **2008**, *26*, 612-621.
2. Nanoparticles in photodynamic therapy: An emerging paradigm, D. K. Chatterjee, L. S. Fong and Y. Zhang, *Advanced Drug Delivery Reviews*, **2008**, *60*, 1627-1637.
3. Nanoparticles in photodynamic therapy, T. Nann, *Nano Biomed. Eng*., **2011**, *3*, 137-143.

<u>Friday after lunch</u>, *topic special*: Photomedicine (Trautinger and Miranda)

Phototherapy: Specific treatment modalities (Trautinger)

The lecture will provide an overview of the various currently used methods of phototherapy and psoralen photochemotherapy. Treatment of psoriasis, atopic dermatitis, cutaneous T-cell lymphomas, vitiligo and other skin diseases will be covered. Special emphasis will be given to extracorporeal photochemotherapy (photopheresis). Methods of radiation delivery, mechanisms of action, clinical results, and adverse reactions will be discussed.
Photopheresis (extracorporeal photochemotherapy)
Franz Trautinger, Ulrike Just and Robert Knobler, Photochem. Photobiol. Sci., 2013, 12, 22-28
DOI: 10.1039/C2PP25144B

Photooxidative reactions of drugs with biomolecules (Miranda)

- Oxidative damage photosentitized by drugs. Type I and type II mechanisms.
- Drug-mediated photooxidation of lipids. Polyunsaturated fatty acids and cholesterol.
- Photoreactivity of drugs with proteins. Oxidation of amino acid residues.
- Covalent photobinding of drug to proteins. Photoantigen formation and its implications in photoallergy.
- Photooxidative DNA damage mediated by drugs. Reactions at the purine bases and at the deoxyribose units.
- Drugs as triplet sensitizers for the photodimerization of pyrimidine bases in DNA.
- Repair of damaged DNA by photoinduced electron transfer. Drugs with potential photolyase activity.

<u>Saturday morning</u>, *topic, basic:*
UV(from cells to skin tissue) (Sage, Tyrrell and Rhodes)

Solar UV-induced DNA damage, repair, mutagenesis and carcinogenesis (Sage)

Solar UV radiation, UVB (295-320 nm) and UVA (320-400 m), is able to induce a plethora of damage types to biomolecules, including DNA, proteins and lipids, with collateral harmful consequences, such as skin aging and carcinogenesis. Acute cellular responses allow to mitigate the long term adverse effects of UV exposure. In this regard, the maintenance of genome integrity which is essential to minimize heritable mutations, for viability of cells and the health of organisms, is insured by surveillance mechanisms such as DNA repair, cell cycle checkpoints and stress signalling cascades. However, when left unrepaired, DNA damage may lead to mutations. DNA damage and mutations represent early genetic events in photocarcinogenesis process. After excessive exposure, massive cell death (apoptosis) may occur and result in peeling of the skin after a few days. This process will prevent heavily damaged cells from contracting mutations. The transcription factor p53 tumor suppressor protein is a key factor in all this signaling network. Its gene has been found mutated in about half of skin tumors and the p53 mutation spectrum in such tumors carries a "UV signature". A UV signature has been observed in a large majority of non-melanoma skin tumours and, recently, through a genome wide analysis, in malignant melanoma as well. The loss of p53 function appears to be an early event in UV carcinogenesis. Other oncogenic pathways are also activated.
The course will 1- define the mechanisms of DNA damage formation, including chemical aspects, 2- present the repair mechanisms available on UV-induced lesions, 3- explain how a DNA lesions which is a transient modification of DNA, can be transformed into a mutation, an heritable change in DNA, 4- provide information on the molecular and genetic aspect of melanoma and non-melanoma skin carcinogenesis.

Special reading, General session:

J. Cadet, E. Sage et T. Douki (2005).Ultraviolet radiation-mediated damage to cellular DNA. Mutat. Res. 571, 3-17.
de Gruijl FR, van Kranen HJ, Mullenders LH. (2001) UV-induced DNA damage, repair, mutations and oncogenic pathways in skin cancer. J Photochem Photobiol B. Oct;63(1-3):19-27.
Bennett DC (2008) Ultraviolet wavebands and melanoma initiation. Pigment Cell Melanoma Res. 21; 520–524
Pfeifer G & Besaratinia A (2012) UV wavelength-dependent DNA damage and human non-melanoma and melanoma skin cancer. Photochemical & Photobiological Sciences, 11 (1), 90-97.

Solar UV generation and biological significance of reactive oxygen species (Tyrrell)

Solar UVA as well as UVB radiations cause damage to skin cells and skin tissue. UVA generates distinct types of damage often associated with oxidative stress and mediated by reactive oxygen species (ROS) which are central to the interaction of UVA with biological material . There are many cellular molecules which absorb UVA and generate reactive oxygen species .Singlet oxygen is undoubtedly the major species generated directly by UVA but other species such as superoxide and hydrogen peroxide are also generated and there are many ways in which these species can interconvert or form the highly diffusible oxidant hydrogen peroxide .UVA also leads to the release of free iron and free heme which are pro-oxidant catalysts and further exacerbate this oxidative stress. A major effect of UVA radiation is to oxidise proteins throughout the epidermis and the dermis and cause damage to the extracellular matrix .Lipids are also oxidised by low levels of UVA and this can set off lipid chain oxidation reactions Such processes can generate many lipid messenger molecules which activate other proteins and enzymes and this includes not only enzymes such as oxidases which generate additional ROS but also sustained activation of proteases which can lead to chronic damage including photaging of human skin

Tyrrell, R.M. (1991) UVA (320-380 nm) radiation as an oxidative stress *in* "Oxidative Stress : Oxidants and antioxidants" (ed. H. Sies), pp 57-83, Academic Press, London.

Solar UVR-induced vitamin D synthesis (Rhodes)

Vitamin D synthesis is the best established beneficial effect of solar UVR exposure. The synthesis of vitamin D is initiated in skin following absorption of UVB by the chromophore 7-dehydrocholesterol (7-DHC, pro-vitamin D), resulting in its photochemical conversion to pre-vitamin D. A slower thermal conversion of pre-vitamin D to vitamin D then follows. Further photochemical reactions occur, of pre-vitamin D and vitamin D to inactive metabolites, in which UVA plays a role. Acquisition of vitamin D through cutaneous synthesis is governed by external and personal factors, the latter being both physiological and behavioural.

This talk will focus on the photobiological aspects of vitamin D, comprising both the accepted scientific theory and recent experimental findings. The pathways for cutaneous synthesis and metabolism will be presented and the action spectrum of pre-vitamin D synthesis will be discussed, including the influence of UVA on this UVB-induced beneficial effect. The influence of the predictable factors of latitude, season and time of day on solar zenith angle, and of the atmospheric variables of ozone, cloud and pollution, which determine the amount of ambient UVB available, will be considered, in addition to personal attributes affecting vitamin D production, including skin pigmentation and age. Recent data on the impact of sun exposure behaviour and photo-protective measures on vitamin D synthesis will be discussed, and will include the length of time of exposure and the skin surface area exposed.

Reading:

Rhodes LE and Webb AR. Ultraviolet radiation and vitamin D. In: Handbook of Organic Photochemistry and Photobiology, 3rd Edition. Eds. Griesbeck A, Oelgemoeller M, Ghetti F. CRC Press, 2012, pp1435-1444

Webb AR. Who, what, where and when - influences on cutaneous vitamin D synthesis. Prog Biophys Mol Biol. 2006; 92: 17-25

Further Reading:

Holick MF, MacLaughlin JA, Clark MB, Holick SA, Potts Jr JT, Anderson RR, Blank IH, Parrish JA, Elias P. Photosynthesis of previtamin D3 in human skin and the physiologic consequences. Science 1980; 210: 203-205

Norval M, Bjorn LO, de Gruijl FR. Is the action spectrum for the UV-induced production of previtamin D3 in human skin correct? Photochem Photobiol Sci 2010; 9: 11–17

Springbett P, Buglass S, Young AR. Photoprotection and vitamin D status. J Photochem Photobiol. B 2010; 101:160-168

<u>Saturday after lunch</u>, *topic special*:
PDT – clinical (Szeimies)

Photodynamic Therapy in Dermatology – From experimental status to routine therapy

Prof. Dr. med. Rolf-Markus Szeimies

Since Hermann von Tappeiner coined the term „photodynamic therapy“ (PDT) in the early 20th century, after treating skin cancer patients with topical dye solutions and subsequent exposure to light, PDT became an important procedure in the treatment of dermatological conditions. Three circumstances made it possible that PDT is meanwhile a success story in dermatology.

1. Topical administration of photosensitizers is the desired way of drug application in dermatology: The use of porphyrin precursors like 5-aminolevulinic acid (ALA) as introduced by Kennedy and coworkers in the early 90ies is possible by topical application on the target lesion, i.e. epithelial tumors like basal cell carcinoma or precancerous lesions (actinic keratoses). ALA or its methyl ester (MAL) penetrates easily abnormal stratum corneum and the underlying epithelial tissue. Due to the need of heme proteins within metabolically active cells, protoporphyrin IX (PPIX) is preferentially synthetized within the tumor. The tumor vs. surrounding tissue ratio is therefore very high (up to 10 in AK, BCC and hyperproliferative diseases like psoriasis vulgaris, acne or human papilloma virus infections), thus enabling a selective destruction of the sensitized tissue.

2. Treatable dermatological diseases are relatively superficial, thus penetration of both photosensitizer and light is possible: Most cancers of epithelial origin are less than 3 mm thick, thus sufficient penetration of hydro- or lipophilic sensitizers down to tumor basis is possible. Besides prior mechanical debulking of protruding tumor parts, current strategies for accumulation of PPIX also consider pretreatments which allow deeper penetration of the drug. For example, physical procedures like the use of CO_2-lasers, mechanical perforation with microneedles (up to 1,500 µm thick), ionto- or phonopheresis can improve PDT efficacy. The use of red light, matching the last absorption band of the porphyrins (Q-band) with a maximum at 630 nm also offers a sufficient activation of porphyrin molecules for a photodynamic reaction even in deeper skin compartiments (up to 3 mm), thus percutaneous illumination is sufficient.

3. For dermatological purposes, simple light sources are adequate for activation of PPIX: For activation of hematoporphyrin derivative and other systemically administered photosensitizers, coherent and monochromatic light is still the golden standard for illumination. This is also necessary to reach hollow organs. For superficial tumors, neither coherence nor monochromasia is required. So, incoherent light sources like filtered lamps, light emitting diodes (LED) or even intense pulsed light matching the absorption spectrum of PPIX can be used. The combination of those light sources (red LED or blue fluorescent lamps) plus either ALA or MAL formulations are registered for the treatment of non-melanoma skin cancer (NMSC), i.e. AK, BCC or Bowen’s disease worldwide. Very recently, daylight-PDT has entered procedural dermatology, i.e. the application of a photosensitizer on field cancerized areas of the body with AK, mostly face and scalp, followed by direct sun exposure. The reason for this approach is amongst others the pain during illumination, which is substantially lower with this approach. However, this is in a way repeating history, since von Tappeiner almost 110 years ago already did the same with his patients.

With registered drugs and light devices, proven efficacy for a variety of dermatological indications and evidence-based guidelines for the use of PDT, this treatment option is an important photobiological procedure for dermatologists not only for oncologic indications but also inflammatory, infectious or aesthetic conditions of the skin.

References:

Babilas P, Schreml S, Landthaler M, Szeimies RM. Photodynamic therapy in dermatology: state-of-the-art. Photodermatol Photoimmunol Photomed. 2010 Jun;26(3):118-32. doi: 10.1111/j.1600-0781.2010.00507.x. Review

Calzavara-Pinton P, Rossi MT, Sala R, Venturini M. Photodynamic antifungal chemotherapy. Photochem Photobiol. 2012 May-Jun;88(3):512-22. doi: 10.1111/j.1751-1097.2012.01107.x. Epub 2012 Mar 1. Review.

Juzeniene A, Peng Q, Moan J. Milestones in the development of photodynamic therapy and fluorescence diagnosis. Photochem Photobiol Sci. 2007 Dec;6(12):1234-45. Epub 2007 Aug 29. Review.

Lee Y, Baron ED. Photodynamic therapy: current evidence and applications in dermatology. Semin Cutan Med Surg. 2011 Dec;30(4):199-209. doi: 10.1016/j.sder.2011.08.001. Review.

Morton CA, Szeimies RM, Sidoroff A, Braathen LR. European guidelines for topical photodynamic therapy part 1: treatment delivery and current indications - actinic keratoses, Bowen's disease, basal cell carcinoma. J Eur Acad Dermatol Venereol. 2013 May;27(5):536-44. doi: 10.1111/jdv.12031. Epub 2012 Nov 26. Review.

Morton CA, Szeimies RM, Sidoroff A, Braathen LR. European guidelines for topical photodynamic therapy part 2: emerging indications--field cancerization, photorejuvenation and inflammatory/infective dermatoses. J Eur Acad Dermatol Venereol. 2013 Jun;27(6):672-9. doi: 10.1111/jdv.12026. Epub 2012 Nov 26. Review.

Ortel B, Calzavara-Pinton P. Advances in photodynamic therapy. A review. G Ital Dermatol Venereol. 2010 Aug;145(4):461-75. Review.

Saturday after lunch, *topic special*:
PDT – preclinical (Berg)

Photochemical internalization (PCI) – from photodynamic targeting of lysosomes to clinical utilization of PCI

The fluorescing properties of photosensitizers can be used to evaluate their intracellular localization and treatment effects. Some photosensitizers localize intracellularly in endocytic vesicles such as endosomes and lysosomes. The treatment effect of light exposure of photosensitizers localized in these vesicles depends on the structure of the photosensitizer. Some photosensitizers may rupture these vesicles without causing substantial damage to the matrix contents of these vesicles. In these cases the contents of these vesicles, e.g. containing endocytoses therapeutics, may be released into the cytosol in a functionally active form that may exert therapeutic effects. This is the basis for the PCI technology. The basic mechanisms and the clinical utilization of this technology will be presented.

Høgset et al. (2004) Photochemical internalisation in drug and gene delivery. *Adv. Drug Deliv. Rev*. 56(1):95-115.

Selbo et al. (2010) Photochemical internalization provides time and space controlled endo-lysosomal escape of therapeutic molecules. J.Control Release. 148: 2-12.

Saturday after lunch, *topic special*:
UV(from cells to skin tissue) (Sage, Tyrrell and Rhodes)

Solar UV-induced DNA damage, repair, mutagenesis and carcinogenesis (Sage)

Solar UV radiation, UVB (295-320 nm) and UVA (320-400 m), is able to induce a plethora of damage types to biomolecules, including DNA, proteins and lipids, with collateral harmful consequences, such as skin aging and carcinogenesis. Acute cellular responses allow to mitigate the long term adverse effects of UV exposure. In this regard, the maintenance of genome integrity which is essential to minimize heritable mutations, for viability of cells and the health of organisms, is insured by surveillance mechanisms such as DNA repair, cell cycle checkpoints and stress signalling cascades. However, when left unrepaired, DNA damage may lead to mutations. DNA damage and mutations represent early genetic events in photocarcinogenesis process. After excessive exposure, massive cell death (apoptosis) may occur and result in peeling of the skin after a few days. This process will prevent heavily damaged cells from contracting mutations. The transcription factor p53 tumor suppressor protein is a key factor in all this signaling network. Its gene has been found mutated in about half of skin tumors and the p53 mutation spectrum in such tumors carries a “UV signature”. A UV signature has been observed in a large majority of non-melanoma skin tumours and, recently, through a genome wide analysis, in malignant melanoma as well. The loss of p53 function appears to be an early event in UV carcinogenesis. Other oncogenic pathways are also activated.
The course will 1- define the mechanisms of DNA damage formation, including chemical aspects, 2- present the repair mechanisms available on UV-induced lesions, 3- explain how a DNA lesions which is a transient modification of DNA, can be transformed into a mutation, an heritable change in DNA, 4- provide information on the molecular and genetic aspect of melanoma and non-melanoma skin carcinogenesis. **The special symposium will be devoted to some specific aspects of UVA.**

Special reading :

Special symposium

Ridley A J, Whiteside J R, McMillan T J and Allison S L (2009) Cellular and sub-cellular responses to UVA in relation to carcinogenesis. International Journal of Radiation Biology 85, 177-195.

Sage E, Girard PM, Francesconi S. (2012) Unravelling UVA-induced mutagenesis. Photochemical & Photobiological Sciences, 11 (1), 74-80.

Schuch et al (2013) DNA damage as a biological sensor for environmental sunlight. Photochemical & Photobiological Sciences, 12 (8), 1259-1272.

Endogenous and exogenous protection against UV generated oxidative stress (Tyrrell)

Homeostatic maintenance of cellular redox state as well as iron and heme levels requires exquisite control to avoid potential cell and tissue damage. Both oxidative (e.g. UVA) damage to cells and tissue as well as inflammatory responses appear to disturb such homeostatic mechanisms and lead to activation of distinct stress responses such as the activation of the antioxidant and anti-inflammatory enzyme, heme oxygenase 1. While DNA repair is crucial to reversal of UVB damage, constitutive endogenous antioxidants (eg glutathione) and constitutive and inducible antioxidant enzymes are major factors in protecting cells and tissue against UVA damage. Various naturally occurring antioxidants have the potential to protect against solar damage. In addition to the antioxidant vitamins, these include carotenoids which scavenge singlet oxygen and phenolic compounds (eg flavonoids) which have strong free radical scavenging properties (by virtue of favourable reduction potentials) and hydroxyl groups which can chelate iron. Substantial information on protection of human cells and skin by a variety of natural antioxidants has accumulated

Reeve,V. and R.Tyrrell (2007). UVA and inducible protection in "Biophysical and Physiological Effects of Solar Radiation on Human Skin" pp 293-310 (ed.P.Giacomoni) RSC press,UK.
Stahl W and Sies H β-Carotene and other carotenoids in protection from sunlight. Am J Clin Nutr.2012 96 1179S-1184S.publications, Cambridge, UK.

Balancing the benefits and risks of solar UVR exposure (Rhodes)

Solar UVR exposure has the well-established beneficial health effect of cutaneous vitamin D synthesis, but also a range of harmful effects including melanoma and non-melanoma skin cancer, photosensitivity and photo-ageing. However, vitamin D is essential for bone health, with low levels causing rickets and osteomalacia and also showing associations with a range of malignant, immune-mediated and systemic disorders. Low amounts of vitamin D are usually obtained through diet, and different recommended levels of oral intake are set in European countries and the USA. On the other hand, vitamin D is efficiently synthesised following relatively short sunlight exposures containing the requisite UVB.

This talk will examine what represents a "healthy" vitamin D status, and how this may be attained and maintained whilst minimising health risks. The findings of recent interventional and observational research and of modelling studies examining UVR dose-vitamin D relationships in humans will be evaluated. This includes examination of winter and summer target vitamin D status and strategies to achieve these. Data will also be compared of the relative efficacy of UVR and vitamin D supplements in raising vitamin D status. Current recommendations on sunlight exposure and vitamin D will be reviewed, with examination of their appropriateness in different population groups.

Reading:

Cancer Research UK SunSmart website http://info.cancerresearchuk.org/healthyliving/sunsmart/

Norval M, Lucas RM, Cullen AP, de Gruijl FR, Longstreth J, Takizawa Y, van der Leun JC. The human health effects of ozone depletion and interactions with climate change. Photochem Photobiol Sci. 2011; 10: 199-225

Further reading:

Farrar MD, Webb AR, Kift R, Durkin MT, Allan D, Herbert A, Berry JL, Rhodes LE. Efficacy of a dose-range of simulated sunlight exposures in raising vitamin D status in South Asian adults: implications for targeted guidance on sun exposure. Am J Clin Nutr. 2013; 97: 1210-1216

Kift R, Berry JL, Vail A, Durkin MT, Rhodes LE, Webb AR. Lifestyle factors including less cutaneous sun exposure contribute to starkly lower vitamin D status in UK South Asians compared to the white Caucasian population. Br J Dermatol. 2013; 169: 1272-8

Webb AR, Kift R, Berry JL, Rhodes LE. The Vitamin D Debate: Translating Controlled Experiments Into Reality For Human Sun Exposure Times. Photochem Photobiol 2011; 87: 741-745.

Poster Abstracts

Effect of Solar Visible, UV-A and UV-B Radiation on the Cyanobacterium, *Microcoleus chthonoplastes*

J. Nana Annan
Department of Biology Education, University of Education, Winneba. Ghana.

ABSTRACT

The effects of solar radiation on photosynthesis, pigments and phycobiliprotein composition were studied in the marine filamentous cyanobacterium, *Microcoleus chthonoplastes* harvested from the intertidal zone of the Biriwa coast in Ghana. The organism was exposed to unfiltered solar radiation (UV-B, UV-A and PAR), and solar radiation filtered through optical filters, WG320 (UV-A and PAR), GG400 (PAR only), and UG5 (only UV-B and UV-A). Photosynthetic oxygen production was impaired by the various components of solar radiation. Unfiltered solar radiation and combined UV-A and PAR had the most severe effect on photosynthesis.

Sucrose gradient ultracentrifugation and absorption spectra of the crude extract of *M. chthonoplastes* indicated chlorophyll *a*, carotenoids, phycoerythrin, phycocyanin and allophycocyanin as the photosynthetic pigments. These pigments were bleached under the various treatments, with phycobilins being affected most. Fluorescence spectra of the pigments had peaks that decreased significantly in amplitude and also shifted towards shorter wavelengths with prolonged exposure time, indicating that energy transfer from the accessory pigments was adversely affected. Sodium dodecyl sulphate - polyacrylamide gel electrophoresis **(**SDS-PAGE) analyses of the protein profile, revealed a loss of high molecular mass proteins and that of low molecular mass (α and β monomers), indicating a dissembling of the phycobilisomal complex and impaired energy transfer from accessory pigments to the reaction centres.

β-Phenyl Quenching of 9-Phenylphenalenone. A novel Photocyclisation Reaction with Biological Implications

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The singlet and triplet excited states of 9-phenylphenalenone undergo β-phenyl quenching (BPQ) via addition of the carbonyl oxygen to the ortho position of the phenyl substituent. This reaction is responsible for formation of the addition product, naphthoxanthene. In the absence of quenchers, naphtoxanthene undergoes a very rapid electrocyclic ring opening reaction reverting to 9-phenylphenalenone. The photoproduct naphtoxanthene also contains a remarkably weak C-H bond (BDEcalc = 37.4 kcal mol^{-1}), which enables efficient hydrogen transfer reactions to suitable acceptors, giving rise to the production of the stable naphthoxanthenyl radical or the naphthoxanthenium cation, depending on the solvent polarity. The reaction shows a remarkable deuterium isotope effect. Photoinduced cyclisation of 9-phenylphenalenone is consistent with the natural occurrence of fluorones in plants.

Photo-inactivation of Bacillus endospores: inter-specific variability of inactivation efficiency

Raquel N. da Silva[1,2], Augusto C. Tomé[1], João P. C. Tomé[1], Maria G. P. M. S. Neves[1], Maria A. F. Faustino[1], José A. S. Cavaleiro[1], Anabela Oliveira[2], Adelaide Almeida[2] and Ângela Cunha[2]

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Bacillus endospores have assumed relevance in medicine and sanitary issues, representing an increased biological risk in relation to vegetative forms. Inactivation of bacterial endospores is a challenge to traditional antimicrobial approaches; therefore, innovative techniques such as photodynamic inactivation (PDI) have been proposed as promising alternatives. In previews works, tricationic porphyrin Tri-Py^{+}-Me-PF, when irradiated with white light, was reported as a highly efficient photosensitizer (PS) for destruction of *Bacillus cereus* endospores.

As a continuation of that work, the following objectives were stablished: (a) evaluate the susceptibility of endospores of *Bacillus cereus*, *B. licheniformis*, *B. sphaericus* and *B. subtilis* to PDI using the same tricationic porphyrin as PS, (b) assesse the efficiency of adsorption of the PS in endospore material as a determinant of the susceptibility of endospores of different *Bacillus* species to photo-inactivation, and (c) determine the value of *B. cereus* as a model organism for studies of PDI of bacterial endospores.

The results of irradiation experiments with endospores of four species of *Bacillus* showed that *B. cereus* was the only species for which efficient endospore photodynamic inactivation (> 3 log reduction) could be achieved. Endospores of *B. licheniformis*, *B. sphaericus* and *B. subtilis* were virtually resistant to photodynamic inactivation with the used tricationic porphyrin. The amount of porphyrin bound to endospore material was not significantly different between species, regardless of the presence of an exosporium or exosporium-like outer layer.

In the light of the results obtained, the sensitivity of endospores to PDI with a tricationic porphyrin is highly variable among different species of the genus *Bacillus*. The presence of an exosporium in endospores of *B. cereus* and *B. sphaericus*, or an exosporium-like glycoprotein layer in endospores of *B. subtilis*, did not affect the amount of bound photosensitizer and did not explain the inter-species variability in susceptibility to photodynamic inactivation. The results imply that the use of *B. cereus* as a more amenable surrogate of the exosporium-producing *B. anthracis* must be carefully considered when testing new PS for their antimicrobial photo-inactivation properties.

References

Hamblin M.R., Hasan T. (2004) *Photochem Photobiol Sci* **3**: 436.

Oliveira A., Almeida A., Carvalho C.M.B., Tomé J.P.C., Faustino M.A.F., Neves M.G.P.M.S., Tomé A.C., Cavaleiro J.A.S., Cunha Â. (2009) *J Appl Microbiol* **106**:1986.

da Silva R. N., Tomé A. C., Tomé J. P. C., Neves M. G. P. M. S., Faustino N. A. F., Cavaleiro J. A. S., Oliveira A., Almeida A., Cunha Â. (2012) *Microbiol Immunol* **56**: 692.

Acknowledgements

Thanks are due to Fundação para a Ciência e a Tecnologia (FCT), European Union, QREN, FEDER and COMPETE for funding the QOPNA research unit (project PEst-C/QUI/UI0062/2013; FCOMP-01-0124-FEDER-037296), and the CESAM (PEst-C/MAR/LA0017/2011). R. N. da Silva thanks FCT for a doctoral fellowship (SFRH/BD/87598/2012). We also thank the European Society for Photobiology for a fellowship to attend the Photobiology School.

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

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

Impact of Skin Colour on Photo-biological Responses

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Abstract

Solar ultraviolet radiation (UVR) is universally acknowledged to be harmful to human health (with the exception of vitamin D formation) and a major risk factor for skin cancer. Epidemiological studies have confirmed an inverse relationship between skin colour and the incidence of skin cancer. This is primarily based on the difference in melanin content in skin of the different skin types. Superficially, melanin content is the greatest difference between different skin colours and its high UVR absorbing nature would suggest that the increased melanin content in dark skin is the reason for the inverse relationship between skin colour and skin cancer. However, it is possible that this inverse relationship is not based solely on the increased overall UVR filtering properties of melanin. Other characteristics of melanin such as its antioxidant nature, radical scavenging properties may be important. Furthermore, there may be differences in the ability to accumulate p53 and DNA repair efficiency, all of which may contribute to the lower skin cancer incidence skin with high levels of melanin.

I am in the early stages of my PhD with a view to studying the photobiology of pigmented skin.

Dispermidined-protoporphyrin IX, New Photosensitizing One Active in Dynamic Phototherapy on Prostate Cancer Cells

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Photodynamic therapy (PDT) using porphyrins as photosensitizers, is approved in the clinical treatment of solid tumors. In the Laboratory of Chemistry of Natural Substances of Limoges University (LCSN), a new strategy of targeting tumor cells has been developed. For this purpose, protoporphyrin IX (PpIX) was vectorized with polyamines such as spermidine, elements preferentially taken up by cancer cells. Phototoxicity of dispermidined-protoporphyrin IX (dSd-PpIX) was evaluated in PC-3 and DU-145 human prostate cancer cell lines. We demonstrated that it was only in case of photoactivation by white light that dSd-PpIX induced apoptosis in these two cell lines. The involvement of caspase-dependent intrinsic pathway has been demonstrated, including a disruption of mitochondrial membrane potential, caspase-9 and caspase-3 activation, cleavage of PARP-1 protein and DNA fragmentation. In addition to this direct apoptotic response, oxidative stress mediated by photoactivated dSd-PpIX induced an increase of cyclooxygenase-2 (COX-2) expression and activity, known as induction of apoptosis resistance. Signaling pathways involved in this phenomenon have also been described, and we have shown that treatment with dSd-PpIX indicated a strong inhibition of the survival pathway NF-κB, and p38/MAPK activation which was known as a pro-apoptotic pathway and inducting of COX-2 expression. Taken together, these data demonstrated that this new photosensitizer could be a good candidate in photodynamic therapy of prostate cancer. These *in vitro* data will be supplemented by *in vivo* experiments based on xenograft models on immunodepressed mice.

Photosensitiser gold nanoparticle conjugates for photodynamic cancer therapy

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Photodynamic therapy (PDT) is becoming increasingly popular for the treatment of cancer. It consists on the excitation of a photosensitiser using visible light (600-700 nm)[1] that, in the presence of molecular oxygen, produces reactive oxygen species, which induce cell death.[2]

Phthalocyanines are widely used photosensitisers. After light excitation and reaction with molecular oxygen, they predominantly produce singlet oxygen ($^1O_2$). The main disadvantage of phthalocyanines is that they are hydrophobic, which limits their delivery and distribution into the cells.[3] For this reason, numerous studies have focused on the use of nanoparticles as delivery vehicles of the photosensitisers into the cells, especially gold nanoparticles.[4,5] Nanoparticles present further advantages as they can be additionally functionalised with other ligands in order to increase selectivity towards cancerous tissue.[3] This is known as targeted PDT.

In this study, gold nanoparticles (*ca.* 4 nm diameter) were synthesised and functionalised with a mixed monolayer of thiolated polyethylene glycol (PEG) and a zinc phthalocyanine (C11Pc) photosensitiser. C11Pc possesses a thiol moiety, which acts as a linker between the C11Pc and the surface of the gold nanoparticles. The gold nanoparticles are further functionalised with anti-HER2 antibody *via* the PEG ligand. This targeting agent was chosen since HER2, human epidermal growth factor receptor 2, is an oncogene overexpressed in more than 25 % of breast cancers.[6]

The synthesised nanoparticle conjugates were characterised using UV-visible and fluorescence spectroscopies. Singlet oxygen production was measured using the probe 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABMA) to prove the conjugates could be used for PDT. In the presence of $^1O_2$, ABMA is photobleached, leading to the formation of its corresponding non-fluorescent 9,10-endoperoxide product.[7] The nanoparticles were used to target SK-BR-3 human breast adenocarcinoma cells, since these cells overexpress the HER2 receptor. The cells were imaged before and after PDT treatment using confocal microscopy. Cell viability assays were also performed to assess the efficacy of the treatment.

(1) El-Sayed, I. H.; Huang, X.; El-Sayed, M. A. *Cancer Lett.* **2006**, *239*, 129.
(2) Van Lier, J. E.; Allen, C. M.; Sharman, W. M. *J. Porphyrins Phthalocyanines* **2001**, *05*, 161.
(3) Obaid, G.; Chambrier, I.; Cook, M. J.; Russell, D. A. *Angew. Chem. Int. Ed.* **2012**, *51*, 6158.
(4) Chatterjee, D. K.; Fong, L. S.; Zhang, Y. *Adv. Drug Delivery Rev.* **2008**, *60*, 1627.
(5) Wang, S.; Gao, R.; Zhou, F.; Selke, M. *J. Mat. Chem.* **2004**, *14*, 487.
(6) Stuchinskaya, T.; Moreno, M.; Cook, M. J.; Edwards, D. R.; Russell, D. A. *Photochem. Photobiol. Sci.* **2011**, *10*, 822.
(7) Wang, M.; Huang, L.; Sharma, S. K.; Jeon, S.; Thota, S.; Sperandio, F. F.; Nayka, S.; Chang, J.; Hamblin, M. R.; Chiang, L. Y. *J. Med. Chem.* **2012**, *55*, 4274.

The influence of cationic galactoporphyrins in protein and lipid oxidation of UV-B resistant bacteria.

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Antimicrobial photodynamic inactivation is becoming a promising alternative to control microbial pathogens. The combination of positively charged groups and carbohydrate moieties with porphyrin derivatives results in an increased cell recognition and water solubility, which improves cell membrane penetration. However, the nature of the oxidative damage and the cellular targets of photodamage are still not clearly identified. This work reports the use of four cationic galactoporphyrins (Fig. 1) as PS against two environmental bacteria, *Micrococcus* sp. and *Pseudomonas* sp., resistant to oxidative stress induced by UV-B exposure. Both bacterial strains were incubated with 0.5 μM (gram positive) and 5.0 μM (gram negative) of photosensitiser and irradiated with white light at a fluence rate of 150 mW cm^{-2} during 15 min. The effect of $^1O_2$ generated during the PDI assays on oxidation of cellular lipids and proteins was assessed. MDA values varied between 0.14 and 3.50 nmol dm^{-3} for *Micrococcu*s sp. and between 0.03 and 0.21 nmol dm^{-3} for *Pseudomonas* sp. after 15 min of PDI treatment. In turn, the analysis of protein carbonyls showed similar variations between for both bacterial strains (between 0.8 and 2.48 nmol cm^{-3}). The occurrence of protein carbonylation and lipid peroxidation supports the hypothesis that antibacterial PDI is triggered by damage of external cell structures such as the cell wall and membrane. This can explain why bacteria considered as resistant to UV-B inactivation can be efficiently photoinactivated in the presence of cationic galactoporphyrin derivatives.

Acknowledgements

Thanks are due to the Portuguese Foundation for Science and Technology (FCT) and FEDER, for funding the QOPNA (project Pest-C/QUI/UI0062/2011) and CESAM research groups, and the Portuguese National NMR Network. This work had financial support from project PTDC/QUI/65228/2006. M. C. Gomes was financed by the BI grant (BI/UI55/4856/2010) and S. Silva is thankful to FCT for her post-doctoral grant (SFRH/BPD/64812/2009). M. C. Gomes is also thankful to ESP photobiology school for her travel grant.

Investigating the photostability and protection offered by mycosporine-like amino acids as sunscreens *in vitro*.

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Background:

Although sunscreens have provided many benefits to human health in reducing sunburn and skin cancer incidence, it has been found that they are potentially damaging to the environment, particularly coastal waters. This has led to a need to find biodegradable sun screening products that are environmentally friendly.

Many marine microorganisms, such as cyanobacteria, contain natural, inbuilt, UVR protection in the form of mycosporine-like amino acids (MAAs) which are produced to protect organisms in environments of high UVR exposure. These compounds have been found in larger fish, particularly in the ocular and epidermal tissues, and eggs. This suggests that these MAAs are accumulated in the food chain and provide protection against UVR to higher species. As well as absorbing UVR, these compounds have been shown to have antioxidant properties.

Research Objectives:

- To investigate the absorbance profile and photostability of the MAA compounds extracted from salmon eggs
- To assess these MAAs as potential sunscreens and antioxidants using the comet assay and comparing this to a commercially available sunscreen with similar UVR absorption characteristics
- To assess MAA as antioxidants using reactive oxygen species (ROS) detection assay.

Methods:

A sample of MAAs extracted from salmon eggs was obtained using a methanol extraction (Confirmed by HPLC & LC-MS). The absorbance profile was determined and then the extract was exposed to increasing doses of solar simulated radiation (SSR) from a solar simulator (conforming to COLIPA standards). The absorbance profile was measured to determine photostability.

Solutions of these MAAs and commercial sunscreens were prepared at different concentrations (0%, 0.1% & 0.3%) and HaCat keratinocytes were irradiated with UVA radiation through these solutions that which were placed on top of the cells. DNA damage was then measured using the comet assay with the use of lesions specific enzymes to look at the specific lesions of cyclobutane pyrimidine dimers (CPD) and 8-oxoGua. A percentage reduction in general and specific DNA damage was then determined by comparing cells irradiated with and without sunscreening agents. Antioxidant ability was detected using the same protocol. The cells were then stained with H_2DCFDA, which fluoresces in the presence of ROS.

Results:

The MAA extract was found to be extremely photostable, even after 60 standard erythema doses (SED), absorbing in the UVB and UVA ranges (290-350nm).

Table 1 shows the results from the comet assay. The salmon egg MAA extract significantly reduced DNA damage (CPDs & 8-oxoGua) due to solar simulated UVA dose dependently, comparable to a commercially available UVA filter. The MAA extract was also shown to have a significant anti-oxidant ability that was not seen by the commercial filter. The reduction in ROS was around 56% ($p = 0.0156$).

Sunscreen	Concentration (w/v)	DNA Lesion	Percentage Reduction	p-value
Salmon Egg extract	0.30%	ALS/SSB	38%	0.0052
		CPD	63%	0.0185
		8-oxoGua	64%	0.0008
UVA Sunscreen	0.30%	ALS/SSB	45%	0.0092
		CPD	42%	0.0225
		8-oxoGua	45%	0.0266

Table 1: Table showing the results of the comet assay.

Conclusions:

In conclusion, MAAs appear to be extremely good photostable candidates to be used as an environmentally friendly sunscreen filter and significantly reduce UVA-induced DNA damage, oxidative damage and are not damaging to costal waters.

MECHANISTIC INSIGHTS IN THE FATE OF SELECTED POLLUTANTS FROM CORK INDUSTRY PHOTOCATALYZED BY TPP^+ AND $TPTP^+$

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The production of cork caps is mainly located in the Iberian Peninsula associated to the strong wine industry in this area. The trees used to produce the cork caps are able to absorb chemicals from the ground. For this reason, it is mandatory to submit the raw cork to a desorption process before manufacturing it to produce the caps. In the typically employed process, boiling water is used to extract the chemicals, and as a result, a cork clean enough for food industry is obtained. However, the wastewater resulting from the washings contains a variety of pollutants whose toxicity precludes the use of biological treatment. Therefore wastewater is often discharged to environment without sufficient decontamination.[1]

Photodegradation processes that use sunlight are being considered as promising tools for partial elimination of contaminants making wastewaters suitable for a further biological treatment.

In this context, herein three representative pollutants from cork industry: gallic acid (GA), pentachlorophenol (PCP) and 2,4,6-trichloroanisole (TCA) have been selected (Fig. 1). Their photodegradation has been investigated using two different photocatalysts, namely 2,4,6-triphenylpyrylium (TPP^+) and 2,4,6-triphenyl(thia)pyrylium ($TPTP^+$) (Fig. 1), acting mainly through oxidative electron transfer mechanisms.[2] Two complementary aspects will be investigated on the photocatalytic treatment of the so-called pollutants. First, their removal under simulated sunlight irradiation will be assessed; secondly, photophysical measurements will be carried out to establish the involved reaction mechanisms.

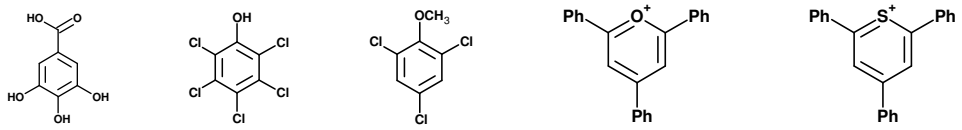

Figure 1: *Chemical structure of the pollutants: Gallic Acid (GA), Pentachlorophenol (PCP) and 2,4,6-Trichloroanisole (TCA) and of the photocatalysts 2,4,6-triphenylpyrylium (TPP^+) and 2,4,6-triphenyl(thia)pyrylium ($TPTP^+$).*

Irradiation of GA, PCP and TCA in the presence of TPP^+ or $TPTP^+$ in organic media resulted in a partial to total removal of the pollutant.

The involvement of the singlet excited state of TPP^+ or $TPTP^+$ in the photodegradation of GA, PCP and TCA was evaluated by time-resolved fluorescence. The singlet lifetime of either TPP^+ or $TPTP^+$ (λ_{exc} = 410 nm or λ_{exc} = 365 nm, respectively) resulted invariable upon increasing concentrations of pollutants, in all cases. Steady-state experiments indicated a decrease in the emission intensity of TPP^+ fluorescence upon increasing concentrations of all pollutants, while when $TPTP^+$ was used as the photocatalyst only in the case of TCA a decrease of the characteristic $TPTP^+$ emission was found. A decrease in the emission of the fluorescence intensity could be an evidence of the formation of a complex between the photocatalyst in its ground state and the pollutant. In fact, the stoichiometry of the complexes was evaluated by Job's Plot and resulted to be 1:1 in all cases. Subsequently, equilibrium constants were determined applying the Benesi Hildebrand protocol.

Finally, participation of the triplet excited state of TPP^+ or $TPTP^+$ was evaluated on the basis of laser flash photolysis experiments. Thus the triplet lifetime of TPP^+ or $TPTP^+$ (λ_{max} = 470 nm for both) was monitored upon increasing concentrations of pollutants. In the case of GA and PCP very efficient quenching constants ($\approx 10^8$ $M^{-1}s^{-1}$) were found, whereas TCA resulted unreactive with $^3TPP^+$ or $^3TPTP^+$.

Overall, the following conclusions can be extracted: photodegradation of pollutants in the presence of the photocatalysts has been observed. The singlet excited states of TPP^+ or $TPTP^+$ do not participate in the photodegradation of the pollutants. TPP^+ is able to form ground-state complexes with the three pollutants, while $TPTP^+$ only forms a complex with TCA. Finally, GA and PCP are able to quench the triplet lifetime of both, TPP^+ or $TPTP^+$**.**

[1] Acero, J., Benitez, J., Beltran de Hereida, J., Leal, A., 2004. Chemical treatment of cork-processing wastewaters for potential reuse*, J. of Chem. Tech. and Biotech*. **79**, 1065–1072.
[2] M.A. Miranda, H. Garcia, 1994. 2,4,6-Triphenylpyrylium Tetrafluoroborate as an Electron-Transfer Photosensitizer, *Chem. Rev*. **94**, 1063–1089.

Determination of vessel depth from hyperspectral images of human hands

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Vascular disorders of the hand are uncommon, but may have lasting implications like pain, color changes in the fingertips, ulcers which do not heal, numbness or tingling of the fingertips and local areas of swelling around the vessels. Vascular problems occur more commonly in individuals with certain diseases such as diabetes, hypertension, or kidney failure, or in dialysis patients. Effective diagnostic and prognostic modalities are needed for early detection and treatment to avoid unnecessary complications and remove symptoms.

Hyperspectral imaging (HSI) is a rapidly growing modality for biomedical applications. The technique provides both spectral and spatial information in one measurement. The main advantages of HSI over conventional techniques are that it is noninvasive, nonionizing, noncontact, and fast. It was previously demonstrated that HIS has an ability to show vessels in human skin.

Hyperspectral signals reflect composition of imaged structure, more explicitly spatial distribution of optical properties. This correlation makes it possible to retrieve depth information from such images. It is well known that deep structures like vessels in the skin appear blue, even though blood is red. The spectra observed at the skin surface are modified depending on scattering and absorption of cromophors in the overlying tissue. We hypothesize that a rough estimate of the depth of vessels can be determined from hyperspectral images.

The aim of the current study is to assess the ability of hyperspectral imaging to evaluate the presence of vessels in human hand and determine the vessel depth. In the first part of the study we measured a phantom developed to determine the depth resolution of HSI. The phantom was composed of a capillary tube filled with fresh, whole blood, immersed diagonally in a container filled with highly scattering solution (10% intralipid in water). This configuration enabled accurate determination of capillary depth at each spatial point of the phantom. We recorded hyperspectral radiance image of the phantom using a high performance push-broom camera (HySpex, NEO, Lørenskog, Norway). The radiance image was converted in a reflectance spectra image, and the latter was analyzed using a light transport numerical model. Hemoglobin concentration and capillary depth were determined at each point of the image and compared to the actual depth of the capillary.

The second part of the study was performed on volunteers. We recorded images of volunteers hands and we are analyzing them using the same methods as for the phantom data. Results of the analysis are positions of vessels and estimation of vessels depths.

Hyperspectral imaging may present a novel, fast, and effective method to diagnose and monitor vascular disorders in hand.

Polyaniline- based Nanostructured Materials for Optical and Electrochemical Formaldehyde sensing

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Abstract

Nowadays nanotechnology attracts great attention and promising future for interdisciplinary and applied science due to the small size and precise characters of the nano-materials. The aim of the current study was to develop an efficient formaldehyde sensor. Polyaniline nanoparticle and gold-polyaniline nanocomposites were prepared, characterized and applied independently as formaldehyde sensors. Formaldehyde sensing was evaluated either optically using microplate assay or electrochemically using potentiostat. It was found that; polyaniline nanoparticle is an efficient sensor whereas it can detect law concentrations of formaldehyde starting from $3x10^{-5}$ ppm. On the other hand, gold-polyaniline nanocomposites showed no significant sensitivity for the detection of law concentrations of formaldehyde. Therefore, polyaniline nanoparticle could be used as a rapid, cheap, stable and sensitive formaldehyde sensor.

Keywords:

Formaldehyde, sensor , gold-polyaniline nanocomposite, optical, electrochemical.

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Photosensitized DNA damage by reactive chlorpromazine metabolites

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Metabolites may be more toxic and reactive than the parent drug. Upon irradiation, they can generate reactive intermediates which can bind to key macromolecules such as DNA. Hence, identification of metabolites with phototoxic capability is a mayor challenge. Chlorpromazine (CPZ), used as an antipsychotic agent, mediates photosensitivity side effects. Thus, the goal of the present work is to evaluate the photogenotoxic potential of chlorpromazine metabolites: desmethylchlopromazine (DMCPZ) and didesmethylchlopromazine (DDMCPZ). To achieve the proposed objective, DMCPZ and DDMCPZ have been synthesized and submitted to photophysical studies in aqueous medium. As in the case of parent CPZ, laser flash photolysis experiments showed the presence of two transient species, triplet and radical cation, with maxima centered at 470 and 520 nm, respectively. Irradiations of CPZ and its metabolites, in the presence of supercoiled DNA, were performed in order to investigate the molecular mechanism involved in UVA-induced DNA damage. Thus, conversion of native supercoiled form I into circular form II was observed, indicating formation of single-strand breaks. In order to notice the nature of damages induced on the DNA bases, repair enzymes (formamidopyrimidine glycosylase, E.coli endonuclease III and T4 endonuclease V) have been used. Interestingly, DMCPZ and DDMCPZ show higher photogenotoxicity than the parent drug CPZ.

Investigating the "By-Stander Effect" of Hypericin-Induced Photodynamic Therapy on *Human Skin Cells*

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A prerequisite for effective cancer treatments is efficient and selective targeting of the tumoral cells with minimal collateral damage to the surrounding normal cells. Whether tumoral cells undergoing treatment respond and/or effect the peri-tumoural cells is called the "bystander effect". We have shown hypericin induced photodynamic therapy (HYP-PDT) to be effective in reducing melanoma cells (Davids et al, 2008). However, PDT's effect on peri-tumoral cells: the normal cells surrounding the tumor, remains intriguing. The aim of this study was to investigate the cellular and molecular effects of HYP-PDT on normal human skin cells, of which cultured primary human keratinocytes, melanocytes and fibroblasts were chosen for this study.

Cell viability analysis revealed a differential response to a range of HYP-PDT doses, in all the human skin cell types, with fibroblasts being the most susceptible, showing a significant difference in cell viability at 1µM (56.40% ± 3.56); 2µM (47.69 % ± 3.89); 3µM (32.94% ± 5.64) and 4µM (33.57% ± 4.72) HYP-PDT. Melanocytes were less susceptible with a significant difference in cell viability at 2µM (60.40% ± 7.14); 3µM (60.83% ± 8.04) and 4µM (38.27% ± 4.27) HYP-PDT. Furthermore, keratinocytes were the least susceptible with an initial significant difference in viability at 4µM (78.95% ± 6.563) HYP-PDT. HYP-PDT susceptibility correlated with intracellular ROS production post treatment: a significant 3.814 ±1.095 fold increase in ROS in fibroblasts, but no significant change in ROS in melanocytes and keratinocytes was observed. Moreover, cellular morphological changes, 24 hours post treatment suggested that these cells are undergoing cell death. Currently, we are investigating different induced modes of cell death, using fluorescence activated cell sorting (FACS). Our results thus far show a variation in response to HYP-PDT by different human skin cells. Future directions include investigating whether the tumor microenvironment affects skin cancer cell response to HYP-PDT and chemotherapy. This therefore contributes to improved targeting of tumor cells and sparing peri-tumoral normal cells.

Synthesis and functionalization of new oxygen sensing porphyrins

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Introduction: Imaging tissue oxygen *in vivo* presents a challenge for regenerative medicine. The successful regeneration of functional tissue relies on the constant supply of oxygen and nutrients.[1] Traditional oxygen detection approaches display a number of disadvantages that make them unsuitable for regenerative medicine. Optical oxygen sensors (OOS) are inexpensive, simple to use, display fast responsiveness and do not suffer from electrical interference nor cause oxygen consumption.[2] OOS has been successfully employed to measure oxygen tension in both 2D and 3D cell cultures. Among the classes of molecules used for oxygen sensing, transition metal porphyrin complexes display high phosphorescence quantum yield and photostability.[3] In addition, the physico-chemical properties of porphyrins can be tuned by structural modification.

Methods: The research is focused on the synthesis of a library of water soluble, conjugatable, oxygen-sensing tetrakis-*meso*-arylporphyrin. The functionalization of the porphyrin provides first of all the introduction of a β-nitro group. Then a Michael-Claisen reaction, followed by an hydrolysis, allows the introduction of a carboxylic group, the anchoring moiety. The subsequent step of the synthesis involve the insertion of a suitable metal ion in the core of the porphyrin ring, to achieve oxygen responsiveness. The most suitable metal ions for this purpose are platinum (II) and palladium (II). The final step of the synthesis will concern modifications to impart water solubility to the molecule. Through this synthetic approach several different compounds, with different physico-chemical properties, can be synthesised starting from the same, single, high-value intermediate.

Result and Discussion: The overall synthetic process presents good yields and a multi-gram scale applicability. The reagent used are cheap and easily available. Moreover, during the whole synthesis, the solvent use is limited and the concentrations are increased, in order reach a greener approach.

Conclusions: A novel synthetic approach has been developed to functionalise porphyrins on the β position. Moreover new water-solubility reactions render the molecules suitable for a bio application. Future development regards the creation of oxygen-sensitive sensors for bio applications.

References:

1. *Capturing complex 3D tissue physiology in vitro. Nat. Rev. Mol. Cell Biol. 7, 211–24 (2006).*

2. *Probes and Polymers for Optical Sensing of Oxygen. Microchim. Acta 143, 1–12 (2003).*

3. *Emerging applications of phosphorescent metalloporphyrins. J. Fluoresc. 15, 569–84 (2005).*

UV-photoexcitation of oxygen encounter complexes X-O_2 as a new source of singlet oxygen $O_2(^1\Delta_g)$

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The photoprocesses in oxygen are very important for chemistry of atmosphere, oxidative organic photochemistry as well as for oxygen assisted photobiochemistry. The photoabsorption of "isolated" oxygen molecules is very weak within the whole UV-IR spectral region where all electronic transitions of ground state O_2 molecule are spin and/or orbital symmetry forbidden. But the interaction of oxygen molecules with molecular environment provides the strong enhancement of UV-absorption by oxygen. This drastic effect is governed by encounter complexes X-O_2 and takes place when molecules colliding in the gas phase and in liquid phase when oxygen is dissolved in some solvent or in solid cryogenic matrixes. This collision-induced enhancement of UV-radiation absorption dramatically changes the oxygen photochemistry resulting in new photochemical processes such as formation of reactive singlet oxygen species $O_2(^1\Delta_g)$. Singlet oxygen molecules possess a high chemical reactivity and play major role in many chemical and biological photooxidation processes such as photosynthesis, mitochondrial respiration, oxidative stress etc.

In recent investigations we have established that the UV-photoexcitation of pure oxygen provides a new channel of singlet oxygen $O_2(^1\Delta_g)$ formation [1] and suggested the possible mechanism of singlet oxygen generation from oxygen encounter complexes O_2-O_2 [2].

In present work the main attention is paid to the qualitative and quantitative description of the mechanism of this new photochemical process proceeding via UV-photoexcitation of O_2-O_2, nitrogen-oxygen N_2-O_2 and isoprene-oxygen C_5H_8-O_2 encounter complexes in the gas phase. In the experiments gas mixtures (pure O_2, N_2+O_2 or C_5H_8+O_2) with oxygen elevated pressure from 2 bar to 150 bar have been excited by laser radiation within the range 238÷285 nm. Singlet oxygen $O_2(^1\Delta_g)$ was observed and detected by its IR-luminescence centered at 1.27 μm. The quantum yield of $O_2(^1\Delta_g)$ molecules photogenerated by the encounter complexes X-O_2 was found to possess rather high maximum value close to 2 at 262.6 nm for X= O_2 and close to 1.5 at 266 nm and at 272 nm for X= N_2, C_5H_8 respectively.

We assume two processes to be responsible for singlet oxygen $O_2(^1\Delta_g)$ formation: due to absorption by individual O_2 molecules and encounter complexes X-O_2 respectively. The process of singlet oxygen formation due to cooperative X-O_2 complex excitation with further simultaneous change of the both partner spins is one of peculiar interest. We suppose that this process may result in singlet oxygen formation in widespread wavelength region including UVA and visible spectral region depending on oxygen collisional partner X. We also assume singlet oxygen generation to follow the excitation of any encounter complexes X-O_2 in any media (gas or condensed) containing oxygen.

The authors gratefully acknowledge the financial support of this work by the Russian Foundation for Basic Research (12-03-00170-a).

[1] A. P. Trushina, V. G. Goldort, S. A. Kochubei, A. V. Baklanov, *Chem. Phys. Letters* **485**, 11 (2010).
[2] A. P. Trushina, V. G. Goldort, S. A. Kochubei, A. V. Baklanov, *J. Phys. Chem. A* **116**, 6621 (2012).

Singlet-oxygen-sensitized delayed fluorescence in living mammalian cells: a time-resolved microscopic approach

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Singlet oxygen ($^1O_2$), the first excited state of molecular oxygen, is a very important reactive oxygen species which readily oxidizes a wide range of biomolecules. As such it is a potent cytotoxic agent. $^1O_2$ is efficiently produced by energy transfer from light-excited states (namely triplet states) of a wide range of chromophores, so-called photosensitizers (PS). The oxidative stress caused by $^1O_2$ can lead to cell death either by necrotic or apoptotic pathway. This is utilized in photodynamic therapy (PDT) of cancer and other diseases where cytotoxic $^1O_2$ is produced after administration of a PS specifically in target tissue, which is illuminated by light of an appropriate wavelength. It is highly desirable to study and understand in detail the processes of formation and deactivation of $^1O_2$ in biological systems. To this end, various techniques for detection and monitoring of $^1O_2$ in cells and tissues have been developed. $^1O_2$ emits a very weak phosphorescence around 1275 nm which allows for its direct detection, but it is experimentally very demanding. Much stronger signal can be provided by indirect methods, such as $^1O_2$ detection by $^1O_2$-sensitive fluorescent probes (e.g. Singlet oxygen sensor green®). However, this requires a separate addition of the probe and various problems may appear, such as non-perfect specificity to $^1O_2$ and production of $^1O_2$ by the probe itself. "Singlet oxygen-sensitized delayed fluorescence (SOSDF)" of a photosensitizer is a process which has been known for decades, but in our opinion its potential for $^1O_2$ detection and monitoring hasn't been fully recognised yet.

The underlying mechanism of SOSDF is an encounter of $^1O_2$ with a triplet state of a PS leading to production of S_1 state of PS, which may then emit a fluorescence photon. Therefore, the PS itself acts as a $^1O_2$ probe. Unlike $^1O_2$ phosphorescence, the SOSDF occurs in visible spectral region, which makes its detection less experimentally demanding and, moreover, SOSDF emission can be up to several orders of magnitude stronger. Upon pulsed laser excitation, the typical rise-decay kinetics of SOSDF can be followed. The lifetimes of SOSDF provide information about both $^1O_2$ lifetime and triplet state lifetime. A wide range of both porphyrin- and non-porphyrin- based water-soluble photosensitizers (TMPyP, $TPPS_4$, $AlPcS_4$, rose bengal, eosin) manifest SOSDF in air-saturated water solutions at physiological pH[1]. SOSDF from a suspension of $TPPS_4$ doped mouse fibroblast cells has also been observed. SOSDF is not limited only to water-based systems. An efficient SOSDF emission was observed for water-insoluble PSs in organic solvents as well (e.g. porphycenes in toluene[2]). These features promote SOSDF as a promising potential new modality for $^1O_2$ monitoring and dosimetry during PDT treatment and for $^1O_2$-based imaging of biological systems.

The contribution presents a microscopic approach to the detection of SOSDF kinetics from living mammalian cells loaded with a photosensitizer. The SOSDF kinetics were collected from area of only ~60×60μm which allows to probe specifically individual cells or small patches of them. SOSDF kinetics from mouse fibroblasts treated with porphyrin-based photosensitizers TMPyP and $TPPS_4$ are shown and the effects of H2O/D2O isotopic exchange, oxygen concentration, and presence of $^1O_2$ quenchers are investigated. SOSDF kinetics from cells have been found to be sensitive to $^1O_2$ and triplet state lifetimes. The work paves the way to further development of techniques of singlet oxygen monitoring by means of SOSDF.

References

1. Scholz M., Dědic R., Hála J. and Nonell S.:, Oxygen effects on tetrapropylporphycene near-infrared luminescence kinetics, J. Mol. Struct., 2013, 1044, 303–307.
2. Scholz M., Dědic R., Breitenbach T., and Hála J.: Singlet oxygen-sensitized delayed fluorescence of common water-soluble photosensitizers, Photochem. & Photobiol. Sci., 2013, 12 (10), 1873 – 188.

Effect of UVA radiation on MCPIP1 expression in HaCaT cells.

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MCPIP1 (Monocyte Chemotactic Protein-1 Induced Protein*)* is a recently identified protein which downregulates the inflammatory response due to its RNase activity to mRNA IL-1β, IL-6 and negative regulation of NF-κB activity upon stimulation with IL-1β and LPS. MCPIP1 is also an important regulator of cell apoptosis but its role in stress response remains unknown.
UVA (320-400 nm) radiation is a well-established oxidizing agent that causes significant damage to cellular components that leads to apoptosis, photoaging and cancer by indirect DNA damage due to reactive oxygen species production (ROS).
Therefore we studied if UVA induces changes of MCPIP1 level in HaCaT cells.
Irradiation of HaCaT cells with UVA (15-20 J/cm^2) increases cell death and caspase 3/7 activity in a dose and time dependent way compared to unirradiated cells. We found that UVA (20J/cm^2) is able to increase MCPIP protein level in HaCaT cells within 6-9 h after the treatment. The underlying mechanisms of MCPIP1 photoxic action require further investigation.
Work supported by the National Science Centre Grant number 2012/05/B/NZ1/00004.

Novel Perioperative Multispectral Imaging Technique

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Several studies demonstrated the capability of diffuse reflectance and autofluorescence point-spectroscopy to improve the sensitivity for detecting various superficial diseases with quantitative information. The physical interactions of photons with the tissue allow the extraction of the optical properties (absorption, diffusion or autofluorescence) corresponding to the tissue chromophores or fluorophores of interest such as oxy- and deoxy- hemoglobin,NADH, flavin, collagen, elastin, etc. Our team has already acquired a large experience in point-spectroscopy techniques to improve the diagnosis of tuberculosis [4], cancer [1] and lung diseases[3], that will benefit this project.

The main challenge in this field is to obtain real-time quantitative images covering a wider field of view than point-spectroscopy. In particular, R. Richards-Kortums team [2] has developed a real-time system with a wide field of view but featuring no quantitative ability; Feld et al.[5] used point-spectroscopy scanning allowing to extract quantitative information but the method is still far from being real-time for the diagnosis of a wide surface.

The aim of our present studies is to develop a novel multispectral imaging technique to obtain quantitative functional and structural information during a real-time image acquisition in order to compose a final 2D quantitative map of a large field of view. It is primarily intended for oral mucosa tumour characterization. Our method is based on non-contact diffuse reflectance and autofluorescence emission measurements in a number of different spectral bands covering a large range from mid-UV to NIR.

References

[1] Amouroux, M., Daz-Ayil, G., Blondel, W. C. P. M., Bourg-Heckly, G., Leroux, A., and Guillemin, F. Classification of ultraviolet irradiated mouse skin histological stages by bimodal spectroscopy: multiple excitation autofluorescence and diffuse reflectance. *Journal of Biomedical Optics 14*, 1 (2009), 014011–014011–14.

[2] Bedard, N., Schwarz, R. A., Hu, A., Bhattar, V., Howe, J., Williams, M. D., Gillenwater, A. M., Richards-Kortum, R., and Tkaczyk, T. S. Multimodal snapshot spectral imaging for oral cancer diagnostics: a pilot study. *Biomedical optics express 4*, 6 (2013), 938–949.

[3] Bourg-Heckly, G., Vever-Bizet, C., Blondel, W., Salan, M., and Thiberville, L. Endoscopic autofluorescence micro-spectroimaging of alveoli: comparative spectral analysis of amiodarone-induced pneumonitis patients and healthy smokers. In *Proc. SPIE* (2011), vol. 7893, pp. 789313–789313–7.

[4] Koenig, A., Grande, S., Dahel, K., Planat-Chrétien, A., Poher, V., Goujon, C., and Dinten, J.-M. Diffuse reflectance spectroscopy: a clinical study of tuberculin skin tests reading. In *Society of Photo-Optical Instrumentation Engineers (SPIE) Conference Series* (Feb. 2013), vol. 8592.

[5] Yu, C.-C., Lau, C., O'Donoghue, G., Mirkovic, J., McGee, S., Galindo, L., Elackattu, A., Stier, E., Grillone, G., Badizadegan, K., Dasari, R. R., and Feld, M. S. Quantitative spectroscopic imaging for non-invasive early cancer detection. *Opt. Express 16*, 20 (Sep 2008), 16227–16239.

Submitted to:
Photobiology 2014

Peptide Targeted Dendrimeric Prodrugs for Aminolevulinic Acid Photodynamic Therapy

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Photodynamic therapy (PDT) is an emerging therapy for the treatment of cancer and various other human disorders, in which targeted destruction of diseased tissue is achieved by exposure to light after selective administration of a light-activated photosensitising drug (photosensitiser). The major advantage of PDT is that the therapeutic effect is highly controlled as it is limited to the illuminated area, and only takes place during illumination, which significantly reduces side effects. 5-aminolevulinic acid (ALA) is a simple natural product that is of great interest for PDT because it can be converted into cells via the haem biosynthetic pathway to a fluorescent photosensitiser- protoporphyrin IX (PpIX) [1]. The aim of this project is to improve the delivery of ALA to specific cell types by developing peptide-targeted ALA prodrugs (Fig. 1). We will describe the synthesis of such molecules in which branched units each with 3 or more copies of ALA [2] are attached to a central core unit using copper-catalysed azide-alkyne click chemistry, along with a "homing" peptide, specific for a cell receptor that is overexpressed by a particular type of cancer (e.g. epidermal growth factor). The peptide conjugates will be evaluated in selected tumour cell lines to assess the enhancement in ALA release/PpIX production compared to non-targeted derivatives.

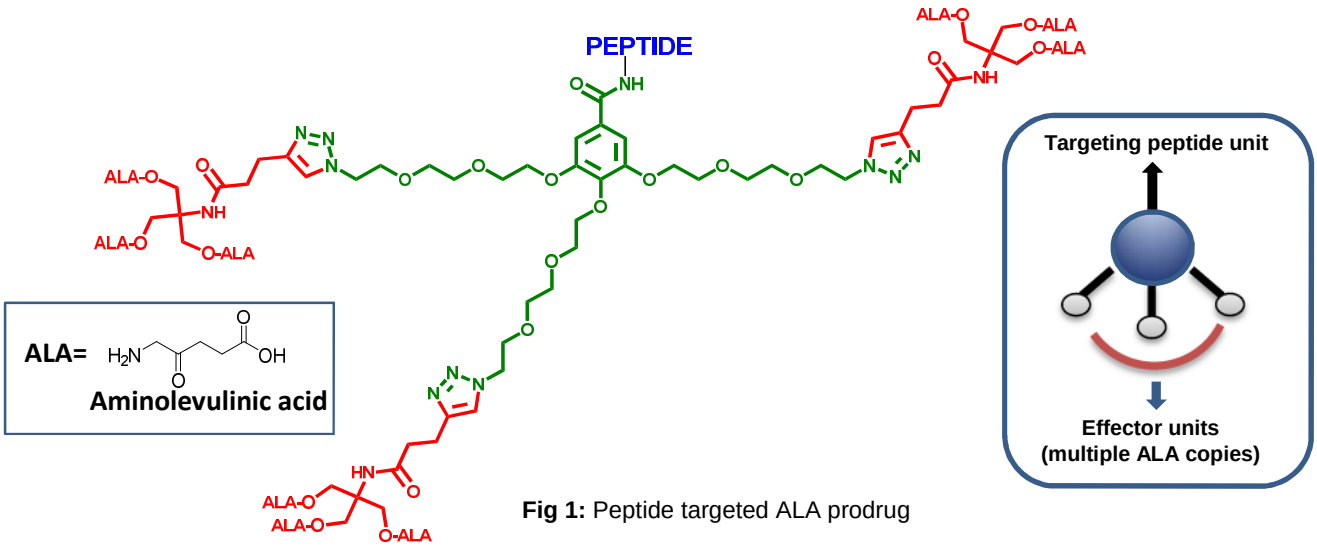

Fig 1: Peptide targeted ALA prodrug

References: [1] Giuntini F, Eggleston IM *et al*. *J. Med. Chem.* **2009**, *52*, 4026; [2] Battah S, MacRobert AJ *et al*, *Mol. Cancer Ther.* **2007**, *6*, 876.

REACHING A MAXIMUM ABSORTTION AT 800 NM THANKS TO OCTA SULFANYL NONPERIPHERAL SUBSTITUTION

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A photosensitiser absorbing in the NIR area of the electronic spectrum has two advantages important in photodynamic therapy:

- light at such wavelengths penetrates deeper the tissues, allowing the treatment of deep tumors
- the absorption occurs in the therapeutic window without affecting biological components.

Since the octa sulfanyl nonperipheral substitution of phthalocyanines is known to shift their maximum absorption up to 800 nm [1-2], our efforts were directed towards the obtention of derivatives with suitable solubity regarding biological applications [3].

The financial support from the Scientific and Technological Research Council of Turkey TUBITAK (project 113Z595) is gratefully acknowledged.

References

[1]Burnham, P. M.; Cook, M. J.; Gerrard, L. A.; Heeney, M. J.; Hughes, D. L. *Chem. Commun*. 2003, 2064–2065

[2] S. Z. Topal, Ü. İşci, U. Kumru, D. Atilla, A. G. Gürek, C. Hirel, M. Durmuş, J. Tomasino, D. Luneau, S. Berber, F. Dumoulin, V. Ahsen, *Dalton Trans*., 2014, 43, 6897–6908

[3] D. A. Tekdaş, U. Kumru, A. G. Gürek, M. Durmuş, V. Ahsen, F. Dumoulin, *Tetrahedron Lett.*, 2012, 53, 5227–5230

SINGLET OXYGEN PHOTOSENSITISATION BY PP2L30M, A NOVEL DERIVATIVE OF *PSEUDOMONAS PUTIDA* FLAVIN-BINDING PP2FBFP

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Flavin binding fluorescent proteins (FbFPs) are a new class of fluorescent reporters that have become a powerful tool for the study of cellular structures and dynamics, providing an exquisite image resolution. The flavin chromophore, which is ubiquitously provided in cellular systems, is tightly bound to the fluorescent protein and rules its spectral and photophysical properties. Flavin's innate high singlet oxygen ($^1O_2$) photosensitisation ability (Φ_Δ=0.51)[1] has stimulated the development of new FbFPs variants with the capacity to sensitise $^1O_2$ as a very useful tool for mechanistic (e.g., chromophore-assisted light inactivation of proteins, CALI) or therapeutic (e.g., photodynamic therapy, PDT) applications.

In this communication we report an investigation on $^1O_2$ photoproduction by PP2L30M, a novel derivative of *Pseudomonas putida* PP2FbFP[2]. Direct detection of the $^1O_2$ near infrared phosphorescence at 1275 nm leads to a Φ_Δ value of 0.09±0.01, which is the highest reported value for a FbFP (ca. 3-fold higher than for miniSOG[3]). Transient absorption experiments revealed two oxygen-dependent decays, suggesting two triplet states, consistent with the kinetics observed for $^1O_2$ phosphorescence. Both triplets contribute very similarly to the $^1O_2$ generation although they greatly differ in their oxygen accessibility. Kinetic models of FbFPs triplet formation and $^1O_2$ production are also discussed. Altogether, this study provides both kinetic and mechanistic approaches of the photosensitising properties of FbFPs that may shed light for the development of new and improved genetically encodable photosensitisers.

[1] Baier, J.; Maisch, T.; Maier, M.; Engel, E.; Landthaler, M. and Bäumler, W; *Biophys. J.*, **2006**, *91*, 1452.

[2] Wingen, M.; Potzkei, J.; Endres, S.; Casini, G.; Rupprecht, C.; Fahlke, C.; Krauss, U.; Jaeger, K.-E.; Drepper, T. and T. Gensch, T.; *Photochem. Photobiol. Sci.*, **2014**, *13*, 875.

[3] Ruiz-González, R.; Cortajarena, A. L.; Mejias, S. H.; Agut, M.; Nonell, S. and Flors, C.; *J. Am. Chem. Soc.*, **2013**, *135*, 9564.

Increased frequency of regulatory T cells with impaired suppressive capacity after PUVA in cutaneous T-cell lymphoma

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Cutaneous T cell lymphoma (CTCL) is a disease of proliferative CD4 T cells homing in the skin that slowly invade lymphoid tissue, peripheral blood and other organs. Psoralen plus UVA (PUVA) photochemotherapy induces apoptosis of malignant cells and is highly effective in the initial stages of the most common CTCL malignancy mycosis fungoides (MF). However, PUVA is also known to induce transient immunosuppression with the up-regulation of CTLA-4, nevertheless, its exact underlying therapeutic mechanism in MF is not yet well understood. We addressed this issue by examining skin biopsies and blood of MF patients before and during PUVA treatment. At baseline we found a low number of $Foxp3^+$ cells in the lymphoid infiltrate in the skin, however, the relative frequency of these cells increased in the remaining infiltrate after several weeks of treatment. The frequency of Treg cells ascended after treatment also in the blood, conversely, their suppressive capacity decayed. Moreover, we saw a decrease of TH17 ($ROR\gamma T^+$) cell frequency and the monitoring of the frequency of malignant cells (measured by expression of CD7 and CD26) in blood did not show a significant difference comparing before and after treatment values, although the patients exhibited remission of lesions. In a mouse lymphoma model we have been able to replicate tumour formation and after PUVA we have observed higher frequencies of Treg cells and lower frequencies of activated CD8 T cells in spleen and lymph nodes. These findings suggest an involvement of Treg cells in the therapeutic mechanisms of PUVA. Whereas PUVA can increase the levels of Treg cells in the blood and skin, the treatment seems to impair Treg function. Taken together, PUVA may normalize the compromised immune surveillance of MF patients.