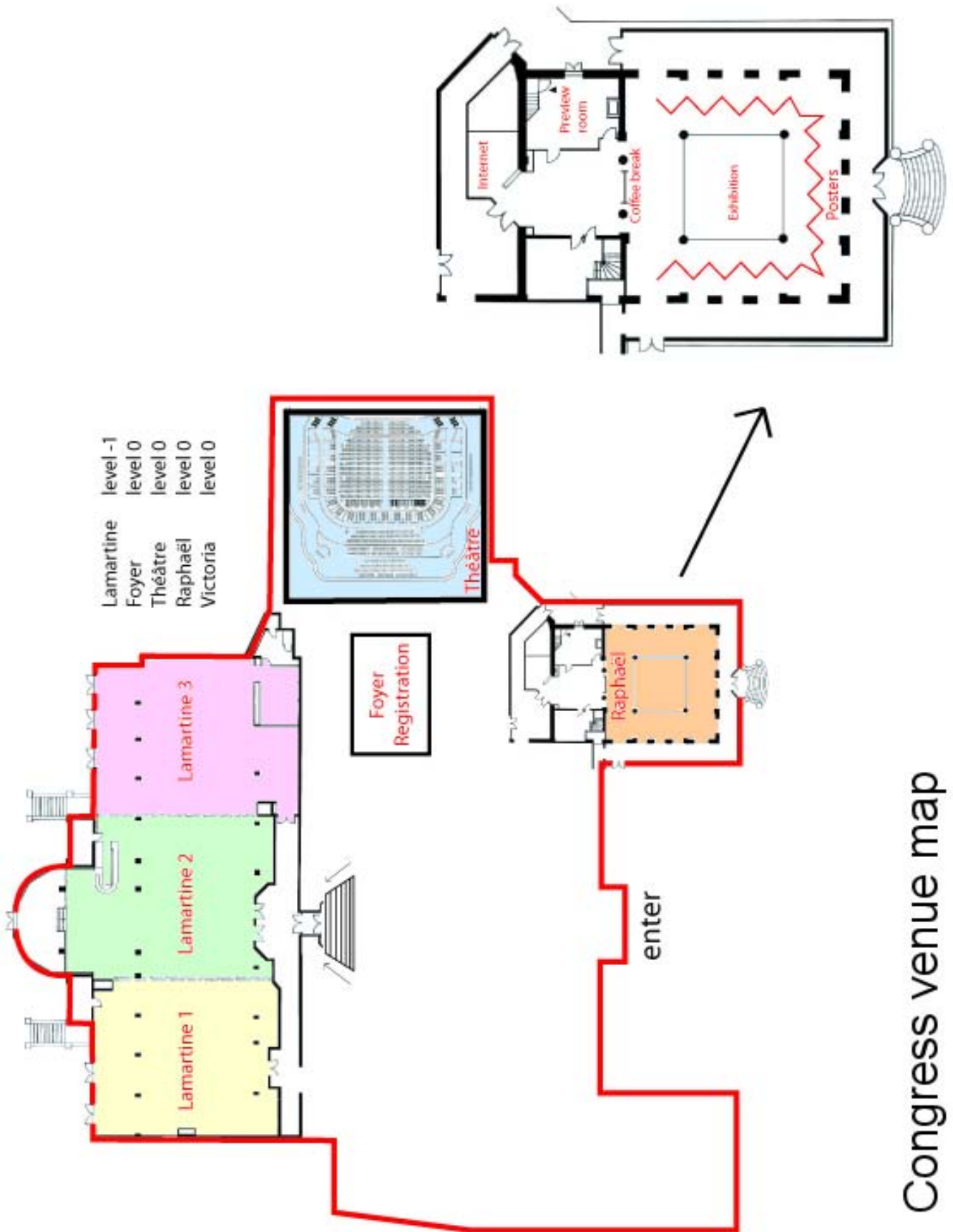


IIth congress
EUROPEAN SOCIETY
FOR PHOTOBIOLOGY

ESP 2005

AIX-LES-BAINS, FRANCE
3 – 8 September 2005

Programme
and
Book of Abstracts



Congress venue map

	Saturday 3	Sunday 4	Monday 5	Tuesday 6	Wednesday 7	Thursday 8
08.45		Th: Opening Ceremony – Welcome Addresses	Th: Photobiology update J. Krutmann: Signal transduction in keratinocytes under UV-A radiation	Th: Photobiology update N. E. Geacintov: Oxidative DNA damage: from electron/hole injection to gel electrophoresis	Th: Photobiology update F. Nagy: Looking for novel circadian clock mutants in <i>Arabidopsis</i>	Th: Photobiology update R. Salimbeni: Photoprotection and photorestitution of cultural heritage
09.00		Th: Young Scientist Award Lecture J.Y. Marroule: NF- κ B, a key player in PDT-induced inflammatory response				
09.30		Coffee Break	Coffee Break	Coffee Break	Coffee Break	Coffee Break
10.00		Parallel Symposia Th: DNA damage and repair L1: ALA-based PDT L2: Oxidative stress in plants L3: Novel blue light receptors	Parallel Symposia Th: Physical chemistry of photosensitizers: membrane interactions, vectorisation and targeting L1: Photodermatology (ESP-D-ESP Joint Symposium) L2: Photodiagnosis and optical techniques for medical diagnosis L3: UV and visible light effects in aquatic environments	Parallel Symposia L1: Photoimmunology (sponsored by L'Oréal) L2: Clinical applications of PDT L3: Light-regulation in plants: growth and rhythms	Parallel Symposia L1: Ocular light damage L2: Cellular response to solar UV L3: Acclimation, stress and regulation of photosynthesis	Parallel Symposia L1: Mechanisms of plant UV-response L2: PDT: oral communications L3: Photochemistry and photobiology of biomolecules
12.00	Executive Committee 2003-2005 (Radisson Hotel)					
14.00		Lunch Break	Lunch Break	Lunch Break	Lunch Break	Lunch Break
14.30		Parallel Symposia Th: Molecular and cellular aspects of PDT L1: Photocarcinogenesis L2: Role of natural and artificial photosynthesis in sustainable energy development L3: Visual pigments and phototransduction (ASP-ESP Joint Symposium)	Parallel Symposia Th: Photoaging L1: UV effects oral communications L2: Photochemistry and photobiology of fullerenes (EPA-ESP Joint Symposium) L3: Photomovements	Parallel Symposia L1: Photoprotection and sunscreens L2: Photochemistry and phototoxicity of drugs L3: Plant photobiology in extreme environments	Parallel Symposia L1: Animal model of photocarcinogenesis L2: Antimicrobial PDT L3: Spectroscopic studies of fast and ultrafast events in photobiology	
15.00	PPS Ownership Board (Radisson Hotel)		Ra: Poster Session I		Ra: Poster Session II	
15.30						
16.00	Registration					
17.30			Th: ESP General Assembly			
18.00	Welcome Reception					
19.00		L1: PPS Editorial Board – Photobiology section			L1: Executive Committee 2005-2007	
20.00		Banquet – ESP Medals Awards (Victoria Hall)				

ROOMS:

Th: Theatre

L1: Lamartine 1

L2: Lamartine 2

L3: Lamartine 3

Ra: Raphaël

Dear Delegates and Friends,

It is my great pleasure to welcome you to the 11th Congress of the European Society for Photobiology in Aix-les-Bains, France.

As in previous ESP congresses, the Scientific Committee has put together a comprehensive programme that consists of photobiology updates, invited lectures, oral communications and poster presentations covering the main fields of photobiology where both fundamental and more applied aspects are addressed. More than 400 contributions will be presented and debated during the congress. The increasing cooperation with several sister societies are illustrated by the organization of joint symposia with the American Society for Photobiology (ASP), the European Photochemistry Association (EPA) and the European Society for PhotoDermatology (ESPD). It is worth noting that we are entering a new era in our relationships with ASP since bi-annual congresses will be now organized alternately in Europe and North America.

We also hope that the delegates and the accompanying persons will have the opportunity to visit and enjoy Aix-les-Bains, the lake and the surroundings including the charming cities of Chambéry and Annecy during or after the congress. A programme of visits will be available for this purpose.

Finally we would like to acknowledge the tremendous efforts deployed by both the Organizing and Local Committees during the last two years to make this congress successful and enjoyable. A special thank to the sponsors, the City mayor for his cooperative action and the Congress office team for its availability and professionalism.

Jean Cadet
Local Chairman

Congress Venue

Casino Grand Cercle
200 rue du Casino
F-73100 Aix-les-Bains, France
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Fax: +33 (0)4 79 88 68 25
Mobile phone: 06 17 92 23 86 or 06 17 92 23 87
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Local Chairman

Dr. Jean Cadet
Laboratory "Lésions des Acides Nucléiques", DRFMC/SCIB, UMR-E CEA-UJF
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Official Language

The official Congress language is English. No simultaneous translation will be available

Hotel Booking Office

Tourist Office of Aix-les-Bains
BP 132
F-73101 Aix-les-Bains CEDEX
Phone: +33 (0)4 79 88 68 00
Fax: +33 (0)4 79 88 68 11
email: reservation.lterraz@aixlesbains.com
<http://www.aixlesbains.com/office-de-tourisme>

Registration

Le Foyer, Casino Grand Cercle
Opening hours Saturday, 3 September: 16.00 – 19.00
 Sunday, 4 September – Wednesday, 7 September: 07.45 – 18.00
 Thursday, 8 September: 07.45 – 13.00

Congress registration fees

	Before June 30	Before August 15	On-site payment
ESP-Members	Euro 350	Euro 400	Euro 500
ESP-Members (Emerging Countries)	Euro 250	Euro 300	Euro 400
ESP-Members (Students)*	Euro 180	Euro 220	Euro 260
Non-ESP-Members**	Euro 500	Euro 550	Euro 650
Accompanying persons	Euro 50 per person		
Social Dinner Tuesday, Sept. 6, 2005	Euro 50 per person		

* Students: Certification letter required.

** For non-ESP members: You may apply for ESP membership at the ESP website.

Registration fee includes: Access to scientific sessions and exhibition hall, Book of abstracts, Coffee breaks, Welcome reception.

Cancellation policy

Please contact the ESP Treasurer: Dr Francesco Ghetti, CNR Istituto di Biofisica, Area della Ricerca di Pisa, Via G. Moruzzi 1, 56100 PISA, ITALY (Telephone: +39 050 3152764, Fax: +39 050 3152760, E-mail: francesco.ghetti@pi.ibf.cnr.it).

All refunds will be processed after the congress.

Please note that 85% of the registration fee will be refunded, but the social dinner fee will not be reimbursed unless cancellation is received before August 25.

ESP-Membership fees for 2005 in EUR

The membership fee includes a subscription to the official journal of the ESP, **Photochemical & Photobiological Sciences (PPS)**, published by the Royal Society of Chemistry (RSC, UK), and available both in electronic and hardcopy versions. Please note that a subscription for more than one year guarantees you a fixed price as well as a reduction on a per-year basis.

Membership including subscription to PPS electronic version for 1 year: **40 EUR (students only)**

Membership including subscription to PPS electronic version for 2 years: **90 EUR**

Membership including subscription to PPS electronic version for 4 years: **170 EUR**

Membership including subscription to PPS electronic and hardcopy version for 1 year: **115 EUR**

Membership including subscription to PPS electronic and hardcopy version for 2 years: **230 EUR**

Membership including subscription to PPS electronic and hardcopy version for 4 years: **450 EUR**

Information for Presenters

• Oral Presentations

Oral presentations will be held in one of the four conference rooms (Theatre, Lamartine 1, Lamartine 2 and Lamartine 3).

• **Plenary lectures:** 40 min presentation (Theatre)

• **Invited lectures:** 30 min presentation including 5 min of discussion (25 + 5 min)

• **Selected oral communications:** 15 min presentation including 3 min discussion (12 + 3 min)

The projection will be via PowerPoint and the operating system is Windows.

Speakers should check for the compatibility of their presentations with PowerPoint 2002 for Windows.

Speakers are invited to upload and check their presentation in a pre-view dedicated room (in the Raphaël area) at least half a day in advance by providing an electronic copy on either a CD or a flash disk/memory stick.

Posters

Posters will be displayed and discussed in the Raphaël exhibition area.

The size of the poster boards is 1.20 m wide x 2 m high.

The posters can be mounted starting Sunday, 4 September and must be removed not later than Thursday, 8 September.

Each poster has been assigned a number (which can be found in the Programme book) and has to be mounted on the poster board with the same number.

Self adhesive tape, holder and string will be available for fixing the poster on the board.

Two poster sessions will be held: on Monday, 5 September and on Wednesday, 7 September, both from 14.00 to 15:30.

The allocation of the posters for the two different sessions can be found in the Programme book.

Presenting authors must be present for the entire duration of the session.

INTERNET connection

An internet point is located in the Raphaël area. Two PC as well as wireless connection will be available.

ESP Awards

During the Banquet the ESP Medals for Excellence in Photobiological Research and the Young Scientist Medal will be awarded.

The recipient of the Young Scientist Medal, Jean-Yves Matroule, will give the Congress opening lecture. The Young Scientist Award is sponsored by the Royal Society of Chemistry, publishers of the ESP official journal, **Photochemical & Photobiological Sciences (PPS)**.

Social events

- Saturday, 3 September, 18.00 - Le Foyer - Welcome reception
- Monday, 5 September, 18.30 - Le Foyer - Wine and cheese tasting
- Tuesday, 6 September, 20.00 - Victoria - Banquet, Medal Awards
- Coffee breaks (from Sunday 4 to Thursday 8) in the Raphaël area

Business Meetings

- Saturday, 3 September, 12.00 - Radisson Hotel - Executive Committee 2003-2005
- Monday, 5 September, 19.00 - Lamartine 1 - PPS Editorial Board - Photobiology section
- Tuesday, 6 September, 17.30 – Theatre - General Assembly
- Wednesday, 7 September, 19.00 - Lamartine 1 - Executive Committee 2005-2007

Programme for accompanying persons

Monday, 5 September: Visit of the City of Aix-les-Bains (15.00 – 17.00) with an English speaking guide. The overall cost for the group visit would be 91 Euro for a maximum of 30 persons.

Wednesday, 7 September: Boat trip on Le Bourget Lake with a visit of the Hautecombe Abbey on the opposite site of the lake. Departure from the harbor at 14.30 with a return scheduled at 17.00. The cost per person is 13.70 Euro.

Visit of the cities of Chambéry (15 km) and Annecy (35 km) can be organized on demand.

Bath therapy at Thermes Chevalley (walking distance from the Congress venue): Access every day from 10.00 until 20.00 at a cost of 15 Euro per person. In the afternoon dedicated spa therapy can be arranged on demand.

Additional information on all these activities will be provided at the registration desk at Casino Grand Cercle.

All Authors are kindly requested to carefully check for mistakes (Authors' names and addresses, title and body of the abstract) and notify them to Francesco Ghetti (francesco.ghetti@pi.ibf.cnr.it) for the final version of the Programme and Book of Abstracts that will be posted on the web after the Congress.

List of Sponsors (as per printing date)

The Argus Center for Photobiology (A Facility of Charles River Laboratories, Inc., Preclinical Services),
Horsham, USA

Centre National de la Recherche Scientifique, France

City of Aix-les-Bains

Commissariat à l'Energie Atomique (Direction des Sciences de la Matière), France

Conseil général de Savoie, France

Conseil régional Rhône-Alpes

Fonds National de la Recherche Scientifique, Belgium

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Navigant Biotechnologies Inc., Belgium

Osato Research Institute, Japan

Pierre Fabre Dermocosmétique, France

Royal Society of Chemistry, Great Britain

Université Joseph Fourier of Grenoble, France

List of Exhibitors (as per printing date)

American Society for Photobiology, USA

ATLAS MTT BV, France

Elsevier BV, The Netherlands

Jobin Yvon, France

Osato Research Institute, Japan

Royal Society of Chemistry, Great Britain

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Thierry Douki	Sandrine Terrier
Olivier Falletti	Jean-Luc Ravanat
Jean-François Millau	

Sunday, September 4, Morning
Theatre

- 08.45 **Opening Ceremony – Welcome Addresses**
- 09.00 **PL1 Young Scientist Award Lecture**
NF- κ B, a key player in PDT-induced inflammatory response
J.-Y. Matroule, C. Volanti, J. Piette (Liège, BEL) *introduced by F. Lenci (Pisa, ITA)*
- 09.30 **Coffee Break**
- DNA damage and repair**
Chair: T. Douki (Grenoble, FRA)
- 10.00 **IL2 Base stacking and base pairing effects on electronic energy relaxation in DNA**
C.E. Crespo-Hernández, B. Cohen, B. Kohler (Columbus OH, USA)
- 10.30 **IL3 Formation and repair of bipyrimidine photoproducts in mammalian cells exposed to UVB and UVA radiations**
T. Douki¹, S. Courdavault¹, E. Sage², C. Baudouin³, M. Charveron³, A. Favier¹, J. Cadet¹
(¹Grenoble, FRA; ²Paris, FRA; ³Toulouse, FRA)
- 11.00 **IL4 Potential role of oxidative DNA damage and repair in the development of malignant melanoma**
S. Hoffmann-Dörr¹, W. Eiberger¹, R. Greinert², B. Volkmer², T.M. Rüniger³, J.P. Radicella⁴, B. Epe¹
(¹Mainz, GER; ²Buxtehude, GER; ³Boston MA, USA; ⁴Fontenay-aux-Roses, FRA)
- 11.30 **IL5 Mechanisms of DNA damage induced inhibition of transcription**
M. Fousteri, D. Arokx, A. Avanzeeland, L.H.F. Mullenders (Leiden, NED)
- 12.00 **OC6 Photochemistry of 5-Halouracil containing DNA**
Y. Xu, H. Sugiyama (Kyoto, JPN)
- 12.15 **OC7 UVB-induced CPD-retaining cells in SKH1-hairless mice are BrdU-retaining stem cells which are NER-proficient**
J.G.W. Nijhof¹, C. van Pelt¹, D.L. Mitchell², W. van Ewijk¹, R. Willemze¹, L.H.F. Mullenders¹,
F.R. de Gruij¹ (¹Leiden, NED; ²Smithville TX, USA)
- 12.30 **OC8 Daily UVB exposure stimulates the global nucleotide excision repair of cyclobutane pyrimidine dimers**
N. Bastien, P.J. Rochette, R. Drouin (Sherbrooke, CAN)

ALA-based PDT*Chair: N. Lange (Geneva, SUI)*

- 10.00 **IL9** **ALA-PDT: the basics**
R. Pottier (Kingston, CAN)
- 10.30 **IL10** **The involvement of the proteasome in regulation of PpIX synthesis and ALA-PDT**
Z. Malik, B. Grinblat, N. Greenberg (Ramat-Gan, ISR)
- 11.00 **IL11** **Prodrug approaches in biomedical optics**
N. Lange (Geneva, SUI)
- 11.30 **OC12** **Photobleaching kinetics and epithelial distribution of hexaminolevulinate induced PpIX in the photodynamic treatment of rat bladder cancer *in vivo***
S. El Khatib, H.P. Lassalle, L. Bezdetnaya, F. Guillemin, M.A. D'Hallewin (Nancy, FRA)
- 11.45 **OC13** **Ultra-sensitive fluorescence imaging of 5-ALA induced Protoporphyrin IX in human glioblastoma cells**
H. Schneckenburger^{1,2}, R. Sailer¹, M. Wagner², H. Emmert¹, W.S.L. Strauss¹ (¹Ulm, GER; ²Aalen, GER)
- 12.00 **OC14** **5-ALA and 5-ALA derivative-mediated effects on gram negative bacteria**
N Fotinos¹, M. Convert², J.-C. Piffaretti², N. Lange¹ (¹Geneva, SUI; ²Bellinzona, SUI)
- 12.15 **OC15** **Photosensitization response measured by reflectance spectroscopy**
P. Juzenas, A. Juzeniene, V. Iani, J. Moan (Oslo, NOR)
- 12.30 **OC16** **Phototoxic activity of 5-aminolevulinic acid-induced protoporphyrin IX against *Leishmania major***
S. Kosaka, O.E. Akilov, T. Hasan (Boston MA, USA)

Sunday, September 4, Morning**Lamartine 2**

Oxidative stress in plants

Chair: K. Apel (Zürich, SUI)

- 10.00 **IL17** **The genetics of ROS-mediated stress responses in *Arabidopsis***
K. Apel (Zürich, SUI)
- 10.30 **IL18** **Crosstalk and antagonistic response to singlet oxygen and other reactive oxygen species in *Arabidopsis thaliana***
C. Laloï¹, A. Baruah¹, E. Pers¹, M. Stachowiak¹, I. Murgia², K. Apel¹ (¹Zürich, SUI; ²Milano, ITA)
- 11.00 **IL19** **Genome wide analysis of photorespiratory hydrogen peroxide regulated gene expression in *Arabidopsis***
F. Van Breusegem, S. Vanderauwera (Gent, BEL)
- 11.30 **IL20** **Molecular biology of programmed cell death in *Arabidopsis thaliana***
T. Gechev^{1,2}, I. Gadjev², L. Bernier¹, M. Zwier¹, M. Ferwerda¹, I. Minkov², J. Hille¹ (¹Haren, NED; ²Plovdiv, BUL)
- 12.00 **OC21** **Ultraviolet illumination induces a stress effect upon higher plants (*Spirodela oligorrhiza*), as evident by the universal stress signal, alanine – an ¹⁵N NMR study**
E. B.-I. Monselise, D. Kost (Beer-Sheva, ISR)
- 12.15 **OC22** **Phototoxic phytoalexins: a new mechanism of light-mediated plant defence**
C. Flors, S. Nonell (Barcelona, ESP)

Novel blue light receptors*Chair: A. Losi (Parma, ITA)*

- 10.00 **IL23** **TbWC-1: the photoreceptor of an hypogeous fungus**
B. Grimaldi, P. Filetici, P. Ballario (Roma, ITA)
- 10.30 **IL24** **Multiple signal chains in the blue light dependent regulation of photosynthesis genes in *Rhodobacter sphaeroides***
H. Happ, Y. Han, S. Braatsch, G. Klug (Giessen, GER)
- 11.00 **IL25** **Signal transduction mechanism of the cryptochrome blue-light photoreceptor**
C.L. Partch, M.W. Clarkson, S. Özgür, A.L. Lee, A. Sancar (Chapel Hill NC, USA)
- 11.30 **IL26** **Effect of mutations on interdomain communication in the phototropin related *Bacillus subtilis* protein Ytva**
A. Losi¹, W. Gaertner² (¹Parma, ITA; ²Mülheim, GER)
- 12.00 **PII82** **Spectroscopic studies on photocycle of the flavin-binding photoreceptor AppA, a bacterial transcriptional anti-repressor of photosynthesis genes**
M. Gauden, S. Yeremenko, W. Laan, I.H.M. van Stokkum, J.A. Ihalainen, R. van Grondelle, K.J. Hellingwerf, J.T.M. Kennis (Amsterdam, NED)
- 12.15 **OC27** **Functional expression of algal light-sensitive adenylyl cyclases in animal cells**
S.V. Schröder-Lang¹, P. Hegemann², M. Watanabe³, G. Nagel¹ (¹Frankfurt, GER; ²Berlin, GER; ³Hayama, JPN)
- 12.30 **OC28** **Light-induced multi-step intra-protein electron transfer in DNA photolyase and cryptochrome**
M. Byrdin¹, M. Vos², A. Eker³, J.P. Bouly⁴, A. Zeugner⁴, B. Giovani^{1,4}, M. Ahmad⁴, K. Brettel¹ (¹Saclay, FRA; ²Palaiseau, FRA; ³Rotterdam, NED; ⁴Paris, FRA)
- 12.45 **PII83** **A blue-light sensing, phototropin-related protein from *Pseudomonas putida*: a paradigm for an extended LOV construct**
U. Krauss¹, A. Losi², W. Gaertner³, K.-E. Jaeger¹, T. Eggert¹ (¹Jülich, GER; ²Parma, ITA; ³Mülheim, GER)

Sunday, September 4, Afternoon
Theatre
Molecular and cellular aspects of PDT
Chair: P. Agostinis (Leuven, BEL)

- 14.30 **IL29** **Molecular targets of photodynamic therapy and cell death pathways: traditional vs. novel linkages between them**
 N.L. Oleinick, L.-Y. Xue, S.-M. Chiu, K. Azizuddin, R.L. Morris, M. Lam, A.-L. Nieminen (Cleveland OH, USA)
- 15.00 **IL30** **Therapeutic exploitation of the tumor-protective mechanisms induced by the photodynamic therapy**
 J. Golab (Warsaw, POL)
- 15.30 **IL31** **Role of endoplasmic reticulum-Ca²⁺ emptying and multidomain pro-apoptotic BAX and BAK proteins in shaping cell death after photodynamic therapy**
 E. Buytaert, G. Callewaert, J.R. Vandenheede, P. Agostinis (Leuven, BEL)
- 16.00 **IL32** **Dissecting cellular and molecular events in PDT in combination with chemotherapy: additivity and synergy**
 A. Chiaviello, E. Crescenzi, G. Palumbo (Napoli, ITA)
- 16.30 **OC33** **Decrease in adhesion of prostate cancer cells following subcurative photodynamic therapy**
 N. Solban, I. Georgakoudi, W. Rice, C. Lin, T. Hasan (Boston MA, USA)
- 16.45 **OC34** **Modulation of cellular Ca²⁺ signalling during PDT**
 G. Pfaffel-Schubart¹, D.E. Bragin², C. Scalfi-Happ¹, C. Hauser¹, A. Uzdensky², A. Rueck¹ (¹Ulm, GER; ²Rostov, RUS)
- 17.00 **OC35** **Photoeffects of zinc(II)-phthalocyanine on cell-substrate adhesion in mouse keratinocytes**
 S. Galaz, J. Espada, F. Sanz, J.C. Stockert, A. Villanueva, M. Cañete, M. Pacheco, V. Moreno, A. Blázquez, A. Juarranz (Madrid, ESP)
- 17.15 **OC36** **Photodynamic injury of isolated crayfish stretch receptor: death of glial cells and neuroglial interactions**
 A.B. Uzdensky, M.S. Kolosov, D.E. Bragin, A.V. Lobanov (Rostov, RUS)
- 17.30 **OC37** **Cell specific effects of polyunsaturated fatty acids on 5-aminolevulinic acid based photosensitization**
 O.A. Gederaas¹, S.A. Schønberg¹, S. Ramstad¹, K Berg², A. Johnsson¹, H.E. Krokan¹ (¹Trondheim, NOR; ²Oslo, NOR)
- 17.45 **OC38** **Detecting apoptosis after PDT: PET imaging with Cu-64 labelled streptavidin following pretargeting of phosphatidylserine with biotinylated annexin-V**
 J.E. Van Lier, N. Cauchon, R. Lecomte, D. Hunting (Sherbrooke, CAN)
- 18.00 **OC39** **Intracellular trafficking of a photosensitizer immunoconjugate using quantum dots: a technique for selective photodynamic therapy**
 D.R. Errabelli, N. Szyncer-Taub, R. Peteranderl, S.K. Chang, B. Ortel, T. Hasan (Boston MA, USA)
- 18.15 **OC40** **Synthesis and *in vitro* testing of photosensitiser-peptide conjugates for use in photodynamic therapy**
 D.I. Vernon, I. Walker, S.B. Brown (Leeds, GBR)

Photocarcinogenesis*Chair: F.R. de Gruijl (Leiden, NED), D. Mitchell (Smithville TX, USA)*

- 14.30 **IL41** **P53 mutations as early events in UV carcinogenesis**
N.M. Wikonkál (Budapest, HUN)
- 15.00 **IL42** **UV-related p53 mutations in human skin carcinoma and precursor stages**
H. Backvall^{1,2}, A. Asplund¹, A. Gustafsson³, A. Sivertsson³, J. Lundeberg³, F. Ponten¹ (¹Uppsala, SWE; ²Boston MA, USA; ³Stockholm, SWE)
- 15.30 **IL43** **Inverse relationship between increased apoptosis and decreased skin cancer in UV-irradiated CD1d^{-/-} mice**
H.N. Ananthaswamy¹, Y. Matsumura², A.M. Moodycliffe³, D.X. Nghiem¹, S.E. Ullrich¹
(¹Houston TX, USA; ²Osaka, Japan; ³Lausanne, SUI)
- 16.00 **IL44** **Effect of DDB2 (Damaged DNA Binding) gene dose on sensitivity to UV carcinogenesis in hairless mice**
S. Alekseev, H. Kool, H. Rebel, M. Fousteri, C. Backendorf, F. R. de Gruijl, H. Vrieling, L.H.F. Mullenders (Leiden, NED)
- 16.30 **OC45** **Oncogenic potential of melanoma associated mutant BRAF**
C.L. Benjamin, V.O. Melnikova, C. Sreevidya, G. Liu, G. Lozano, H.N. Ananthaswamy (Houston TX, USA)
- 16.45 **OC46** **Molecular analysis of risk factors associated with skin cancers from immunosuppressed renal transplantation patients**
S. Queille¹, L. Luron¹, A. Spatz¹, M.F. Avril¹, V. Ribrag¹, P. Duvillard¹, C. Hiesse², A. Sarasin¹, J.P. Armand¹, L. Daya-Grosjean¹ (¹Villejuif, FRA; ²Le Kremlin Bicêtre, FRA)
- 17.00 **OC47** **Loss of expression of CDKN2A via deletion and promoter hypermethylation in human non melanoma skin cancers**
A. Pacifico¹, G. Leone¹, M. Picardo¹, H.N. Ananthaswamy² (¹Roma, ITA; ²Houston TX, USA)
- 17.30 **OC48** **The increase in melanoma: are dietary photosensitizers responsible?**
R.M. Sayre (Memphis TN, USA)

Sunday, September 4, Afternoon**Lamartine 2**

Role of natural and artificial photosynthesis in sustainable energy development*Chair: A.L. Moore (Tempe AZ, USA)*

- 14.30 **IL49** **The Photosystem II reaction centre of *Acaryochloris marina*, a predominantly chlorophyll d containing cyanobacterium**
A. Telfer¹, M. Chen², A. Pascal³, A.W.D. Larkum², J. Barber¹ (¹London, GBR; ²Sydney, AUS; ³Gif-sur-Yvette, FRA)
- 15.00 **IL50** **Biomimicking photochemical reactions in artificial photosynthetic complexes**
A. Magnuson¹, N. Shaikh¹, O. Johansson², M. Borgström¹, J. Höglblom³, W. Shi¹, P. Huang¹, R. Lomoth¹, M. Anderlund², L. Sun², L. Hammarström¹, S. Styring¹ (¹Uppsala, SWE; ²Stockholm, SWE; ³Lund, SWE)
- 15.30 **IL51** **Biomimetic models of Photosystem II driven by ruthenium complexes**
F. Lachaud¹, A. Quaranta², Y. Pellegrin¹, P. Dorlet¹, M.-F. Charlot¹, S. Un², W. Leibl², A. Aukauloo¹ (¹Orsay, FRA; ²Gif-sur-Yvette, FRA)
- 16.00 **IL52** **Bioinspired energy conversion schemes**
M. Hambourger, D. Gust, T. Moore, A.L. Moore (Tempe AZ, USA)
- 16.30 **IL53** **The photochemistry of novel flavin-based photoreceptors**
J. Kennis (Amsterdam, NED)

**Visual pigments and phototransduction
(Joint with the American Society for Photobiology)***Chair: R.K. Crouch (Charleston SC, USA), D.-P. Häder (Erlangen, GER)*

- 14.30 **IL54** **Effects of ultraviolet on vision and the human eye – acute and delayed**
D.H. Sliney (Gunpowder MD, USA)
- 15.00 **IL55** **Effects of sunlight on the human eye – the ophthalmohelioses**
A. Cullen (Waterloo, CAN)
- 15.30 **IL56** **Production and clearance of all-trans retinol in bleached rod and cone photoreceptors**
P. Ala-Laurila¹, M. Estevez¹, R.K. Crouch², Y. Koutalos², B. Wiggert³, M.C. Cornwall¹
(¹Boston MA, USA; ²Charleston SC, USA; ³Bethesda MD, USA)
- 16.00 **IL57** **The role of the retinoid cycle in the visual process**
R.K. Crouch, B. Rohrer (Charleston SC, USA)
- 16.30 **IL58** **Visual pigment coexpression. A means of color cone development and regeneration?**
Á. Lukáts, Á.I. Berta, G. Halász, A. Szabó, Á. Szél (Budapest, HUN)
- 17.00 **PII73** **Late stages of photolysis: cone vs. rod visual pigments**
E.Yu. Golobokova, V.I. Govardovskii (St. Petersburg, RUS)
- 17.30 **PII74** **Light induced melatonin suppression – indications for a dose dependence**
K. Schulmeister¹, M. Weber¹, E. Schernhammer² (¹Seibersdorf, AUT; ²Boston MA, USA)

Monday, September 5, Morning**Theatre**

- 08.45 **PL59 Photobiology Update**
Signal transduction in keratinocytes under UV-A radiation
 J. Krutmann (Duesseldorf, GER) *introduced by J. Piette (Liège, BEL)*
- 09.30 **Coffee Break**
- Physical chemistry of photosensitizers: membrane interactions, vectorisation and targeting**
Chair: D. Brault (Paris, FRA), B. Ehrenberg (Ramat-Gan, ISR)
- 10.00 **IL60 Photosensitization in a confined membranal phase: uptake, topography and photochemistry of sensitizers in membranes**
 B. Ehrenberg (Ramat-Gan, ISR)
- 10.30 **IL61 Steady-state and dynamic interactions of photosensitizers with plasmatic proteins and membranes**
D. Brault, S. Bonneau, H. Mojžišová, C. Vever-Bizet (Paris, FRA)
- 11.00 **IL62 Distribution properties of hematoporphyrin in the mitochondrial permeability transition pore**
F. Ricchelli¹, S. Gobbo¹, G. Jori¹, V. Petronilli¹, P. Nikolov² (¹Padova, ITA; ²Sofia, BUL)
- 11.30 **IL63 Towards a targeted photodynamic therapy**
J. Blais¹, P. Maillard², V. Sol³, P. Krausz³ (¹Paris, FRA; ²Orsay, FRA; ³Limoges, FRA)
- 12.00 **OC64 Enhanced selectivity of tri-component pro-drugs: initial *in vitro* and *in vivo* results**
 E. Dickson, R. Goyan, J. Kennedy, K. Latulippe, R. Pottier, J. Wojtyk (Kingston, CAN)
- 12.15 **OC65 Proteolytically-induced photosensitization, a new tool in photodynamic therapy**
M.A. Campo, D. Gabriel, N. Lange, R. Gurny (Geneva, SUI)
- 12.30 **OC66 Effects of photosensitizers nanoencapsulation and of the size of the nanoparticles on photothrombic activity for the treatment of choroidal neovascularisation by photodynamic therapy**
B. Pegaz, E. Debeve, J.-P. Ballini, Y. Konan, H. van den Bergh (Lausanne, SUI)
- 12.45 **OC67 Vectorisation of photosensitisers (protoporphyrin IX and hypericin) by vesicles expelled by *Dictyostelium discoideum* cells: a new vectorisation tool for cancer diagnosis and photodynamic therapy (PDT)?**
I. Tatischeff, F. Laviaille (Evry, FRA)
- 13.00 **OC68 Photodynamic activity of an estrogen-pheophorbide (a) conjugate in human breast cancer cells (MCF7)**
 N. El-Akra, J.-P. Souchard (Toulouse, FRA)

Photodermatology**(Joint with the European Society of PhotoDermatology)***Chair: J.-C. Béani (Grenoble, FRA), R. Roelandts (Leuven, BEL)*

- 10.00 **IL69** **Anti-oxidants and natural UV filters in skin protection**
F. Boehm (Berlin, GER)
- 10.30 **IL70** **What's new in sunscreens**
G.M. Murphy (Dublin, IRL)
- 11.00 **IL71** **What's new in the photodermatoses**
J. Hawk (London, GBR)
- 11.30 **IL72** **Photodynamic therapy in dermatology: recent advances**
C. Bédane (Limoges, FRA)
- 12.00 **OC73** **Photodynamic action of red light for treatment of erythrasma. Preliminary results**
S. Darras, O. Carpentier, P. Vincent, A. Bonnevalle, P. Thomas (Lille, FRA)
- 12.15 **OC74** **Porphyrin photosensitizers, suitable for the photodynamic treatment of tinea infections caused by *Trichophyton rubrum*?**
T. Smijs, H.J. Schuitmaker, J.A. Bouwstra, S. Pavel (Leiden, NED)
- 12.30 **OC75** **Psoralen metabolism and psoralen ultraviolet-A (PUVA) sensitivity: a role for cytochrome P450 CYP1B1**
G. Smith, Y.Y. Deeni, J. Woods, J. Ferguson, C.R. Wolf, S.H. Ibbotson (Dundee, GBR)
- 12.45 **OC76** **Differential impact of XPD gene mutations associated with either the photosensitive cancer-free trichothiodystrophy or the cancer-prone xeroderma pigmentosum syndrome**
V. Bergoglio¹, L. Riou², O. Chevalier-Lagente¹, A. Sarasin¹, T. Magnaldo¹ (¹Villejuif, FRA; ²Fontenay-aux-Roses, FRA)
- 13.00 **OC77** **Mitochondrial damage and G1 arrest are the primary events for PUVA-induced apoptosis in human keratinocytes**
G. Viola, E. Fortunato, D. Vedaldi, L. Del Giudice, S. Disarò, G. Basso, F. Dall'Acqua (Padova, ITA)

Monday, September 5, Morning

Lamartine 2

Photodiagnosis and optical techniques for medical diagnosis*Chair: P. Taroni (Milano, ITA)*

- 10.00 **IL78** **Development of fluorescence cystoscopy with Hexvix®: the road from *in vitro* studies to clinical results**
H. van den Bergh¹, P. Jichlinski¹, D. Aymon¹, A. Marti¹, N. Lange², L. Guillou¹, P. Kucera¹, H.-J. Leisinger¹, G. Wagnières¹ (¹Lausanne, SUI; ²Geneva, SUI)
- 10.30 **IL79** **Time domain optical mammography and spectroscopy of the breast**
P. Taroni, A. Pifferi, L. Spinelli, A. Torricelli, G. Danesini, R. Cubeddu (Milano, ITA)
- 11.00 **IL80** **Studying brain physiology and pathology with non-invasive near infrared spectroscopy**
J. Steinbrink (Berlin, GER)
- 11.30 **IL81** **High speed optical coherence tomography in ophthalmology**
M. Wojtkowski, I. Gorczyńska, T. Bajraszewski, M. Szkulmowski, A. Szkulmowska, P. Targowski, A. Kowalczyk (Torun, POL)
- 12.00 **OC82** **Autofluorescence characterization of human steatotic liver**
A.C. Croce¹, U. Bareato², D. Neri², U. Cillo², I. Freitas¹, G. Bottiroli¹ (¹Pavia, ITA; ²Padova, ITA)
- 12.15 **OC83** **Influence of cholesterol on cell membrane dynamics probed by fluorescence spectroscopy and imaging**
P. Weber¹, H. Schneckenburger^{1,2}, M. Wagner¹, W.S.L. Strauss² (¹Aalen, GER; ²Ulm GER)
- 12.30 **OC84** **Comprehensive study of the phenomenological mechanisms involved in autofluorescence bronchoscopy**
T. Gabrecht, P. Uehlinger, T. Glanzmann, S. Andrejevic, P. Grosjean, A. Radu, P. Monnier, H. van den Bergh, G. Wagnières (Lausanne, SUI)
- 12.45 **OC85** **Laser induced delayed luminescence from normal fibroblasts and melanoma cells: spectral analysis as a non-invasive diagnostic tool**
L.A. Applegate¹, F. Musumeci², H.J. Niggli³, A. Scordino², S. Tudisco² (¹Lausanne, SUI; ²Catania, ITA; ³Treyvaux, SUI)

UV and visible radiation effects on aquatic environments*Chair: F. Ghetti (Pisa, ITA)*

- 10.00 **IL86** **Interactive effects of UVR and mixing in aquatic environments**
E.W. Helbling, V.E. Villafañe (Playa Unión, ARG)
- 10.30 **IL87** **Photoreceptors in marine diatoms**
M. Mangogna¹, S. Coesel¹, M. Siaut¹, F. Maumus², A. Falciatore¹, A. De Martino^{1,2}, C. Bowler^{1,2}
(¹Napoli, ITA; ²Paris, FRA)
- 11.00 **IL88** **Synthesis and accumulation of mycosporine-like amino acids: an essential strategy to minimize UV damage in freshwater organisms**
R. Sommaruga (Innsbruck, AUT)
- 11.30 **IL89** **Models and molecular markers for UV-B stress evaluation in aquatic ecosystems**
R. Marangoni, D. Gioffré, G. Colombetti (Pisa, ITA)
- 12.00 **OC90** **Impact of solar UV radiation on bacterioplankton and phytoplankton biomasses and activities at different depths in NW Mediterranean coastal water during summer**
F. Joux¹, I. Obernosterer¹, P. Conan¹, F. Lantoine¹, W.H. Jeffrey², A. Baldwin²
(¹Banyuls-sur-mer, FRA; ²Pensacola FL, USA)
- 12.15 **PII80** **Solar UV radiation modulates daily production and DNA damage of marine bacterioplankton from a productive upwelling zone (36°S), Chile**
K.L. Hernández¹, R.A. Quiñones¹, G. Daneri^{1,2}, M.E. Farias³, E.W. Helbling⁴ (¹Concepción, CHI; ²Valparaíso, CHI; ³San Miguel de Tucumán, ARG; ⁴Playa Unión, ARG)

Monday, September 5, Afternoon

Theatre**Photoageing***Chair: K. Scharffetter-Kochanek (Ulm, GER)*

- 15.30 **IL91** **The role of mitochondria in photoaging and aging**
M. Berneburg (Tübingen, GER)
- 16.00 **IL92** **Changes in elastin and microfibrillar component in photoaging**
J.H. Chung (Seoul, KOR)
- 16.30 **IL93** **Epidermal-dermal interactions in photoaging and photocarcinogenesis**
F. Bernerd¹, M. Frechet², A. Sarasin², T. Magnaldo² (¹Clichy, FRA; ²Villejuif, FRA)
- 17.00 **IL94** **Overexpression of a putative ubiquitin c-hydrolase isolated from PUVA-senesced fibroblasts results in a senescence-like phenotype**
Y. Chen, M. Wlaschek, C. Hinrichs, W. Ma, N. Gall, K. Scharffetter-Kochanek (Ulm, GER)
- 17.30 **OC95** **Free-electron laser photoelectron emission microscopy of human pigments**
J.D. Simon¹, L. Hong¹, G.S. Edwards¹, R.J. Nemanich², J. Garguilo² (¹Durham NC, USA; ²Raleigh NC, USA)

UV effects: oral communications*Chair: J. Piette (Liège, BEL)*

- 15.30 **OC96** **Ultraviolet-B radiation inhibits local T cell activation and systemically induces T cell migration into the liver**
S. Rana, S.N. Byrne, L.J. MacDonald, G.M. Halliday (Sydney, AUS)
- 15.45 **OC97** **Epidermodysplasia verruciformis human papillomavirus types are associated with squamous cell carcinomas of xeroderma pigmentosum patients**
L. Luron¹, M.F. Avril¹, B. Bouadjar², A. Sarasin¹, L. Daya-Grosjean¹ (¹Villejuif, FRA; ²Algers, ALG)
- 16.00 **OC98** **Examination of solar ultraviolet radiation exposure of road construction workers in Austria**
M. Weber¹, M. Schwaiger¹, K. Schulmeister¹, H. Brusl², P. Kindl³, P. Knuschke⁴ (¹Seibersdorf, AUT; ²Vienna, AUT; ³Graz, AUT; ⁴Dresden, GER)
- 16.15 **OC99** **Deficient inflammatory response in neonatal mouse skin may contribute to the susceptibility of HGF/SF transgenic mice to UV-induced melanoma**
A. Wolnicka-Glubisz, E. De Fabo, F.P. Noonan (Washington DC, USA)
- 16.30 **OC100** **Ultraviolet-induced ($\lambda=254\text{nm}$) degradation of the bacteriophage MS2 genome (ssRNA)**
J. Simonet, C. Gantzer (Nancy, FRA)
- 16.45 **OC101** **Diastereoselectivity and site-dependency in the photochemistry of ketoprofen in the bovine serum albumin matrix**
S. Monti¹, I. Manet¹, F. Manoli¹, S. Sortino² (¹Bologna, ITA; ²Catania, ITA)
- 17.00 **PI32** **Induction of CCL21/SLC and dendritic cell activation by photodynamic therapy**
S.O. Gollnick, B. Owczarczak, B.W. Henderson (Buffalo NY, USA)
- 17.15 **PI33** **Induction of tolerance by UV-induced, platelet activating factor-stimulated, IL-10 secreting, B cells**
Y. Matsumura, D.X. Nghiem, Y. Miyahara, S.N. Byrne, S.E. Ullrich (Houston TX, USA)
- 17.30 **PI35** **Directing the immune system by light**
C.H. Self, M.-C. Fawcett, A. Self, J.A. Smith, S. Thompson (Newcastle-upon-Tyne, GBR)
- 17.45 **PI34** **The effects of TL01 phototherapy on UVB-induced Langerhans' cell, CD11b+ and iC3b+ cell trafficking in polymorphic light eruption (PLE)**
A. Blackburn, S. Winhoven, M. Brownrigg, L.E. Rhodes, N.K. Gibbs (Manchester, GBR)

Monday, September 5, Afternoon**Lamartine 2**

**Photochemistry and photobiology of fullerenes
(Joint with the European Photochemistry Association)***Chair: R. Bensasson (Paris, FRA), G. Orlandi (Bologna, ITA)*

- 15.30 **IL102** **Environment effects on the spectroscopy and intramolecular relaxation processes of C₆₀**
M. Chergui (Lausanne, SUI)
- 16.00 **IL103** **Organic and hybrid [60]-fullerene multicomponent architectures**
N. Armaroli, G. Accorsi, J.N. Clifford, Y. Rio (Bologna, ITA)
- 16.30 **IL104** **Molecular engineering in artificial photosynthesis**
H. Imahori (Kyoto, JPN)
- 17.00 **IL105** **From photosynthesis to photonic molecular switches based on fullerenes**
D. Gust, T.A. Moore, A.L. Moore (Tempe AZ, USA)
- 17.30 **IL106** **Sequence-specific DNA photocleavage and potential applications**
A.S. Boutorine¹, T. Da Ros², M. Prato² (¹Paris, FRA; ²Trieste, ITA)
- 17.00 **IL107** **Radical scavenging by C₆₀ in model systems and inhibition by C₆₀ of hepatotoxicity induced by CCl₄ *in vivo***
F. Moussa (Chatenay Malabry, FRA)

Monday, September 5, Afternoon**Lamartine 3**

Photomovements*Chair: K.J. Hellingwerf (Amsterdam, NED)*

- 15.30 **IL108** **Microbial rhodopsins: structure and mechanism in photomotility**
J.L. Spudich, E.N. Spudich, J. Sasaki, C.-S. Yang (Houston TX, USA)
- 16.00 **IL109** **Roles for photoactivated adenylyl cyclase (PAC) in photocontrol of flagellar movement in euglenoid cells**
M. Watanabe^{1,4}, S. Matsunaga¹, S. Yoshikawa², M. Iseki^{3,4} (¹Hayama, JPN; ²Obama, JPN; ³Kawaguchi, JPN; ⁴Okazaki, JPN)
- 16.30 **IL110** **Phototaxis and signal transduction in the cyanobacterium *Synechocystis* sp PCC6803**
D. Bhaya, J.T. Ross, F. Fazeli, M.S. Burriesci (Stanford CA, USA)
- 17.00 **IL111** **A balanced purple-bacterial motility response to illumination**
K.J. Hellingwerf¹, M.A. van der Horst¹, J.P. Armitage², M. Roberts² (¹Amsterdam, NED; ²Oxford, GBR)
- 17.30 **OC112** **Signal perception and response in benthic diatom photomovement**
D. McLachlan^{1,2}, A.R. Taylor², R.J. Geider¹, G.J.C. Underwood¹, C. Brownlee² (¹Colchester, GBR; ²Plymouth, GBR)

Tuesday, September 6, Morning**Theatre**

- 08.45 **PL113 Photobiology Update**
Oxidative DNA damage: from electron/hole injection to gel electrophoresis
N.E. Geacintov, V. Shafirovich (New York NY, USA) *introduced by J. Cadet (Grenoble, FRA)*
- 09.30 *Coffee Break*

Tuesday, September 6, Morning**Lamartine 1****Photoimmunology***Chair: J. Krutmann (Duesseldorf, GER)*

- 10.00 **IL114 UVB and UVA wavelengths target different skin compartments: implications for photo-immunology**
F. Bernerd (Clichy, FRA)
- 10.30 **IL115 A role for Platelet Activating Factor receptor binding in UV-induced immune suppression and skin cancer induction**
S.E. Ullrich (Houston TX, USA)
- 11.00 **IL116 The osmolyte taurine plays a critical role in ultraviolet B radiation-induced immunosuppression**
N. Schade¹, C. Esser¹, I. Felsner¹, U. Warskulat¹, A. Schwarz², T. Schwarz², S. Grether-Beck¹, D. Häussinger¹, J. Krutmann¹ (¹Duesseldorf, GER; ²Kiel, GER)
- 11.30 **IL117 UVA induced immunosuppression**
D. Moyal, A. Fourtanier (Clichy, FRA)
- 12.00 **OC118 UV-A-induced immunosuppression plays a role in UV-A induced melanoma metastasis in mice**
R. Pastila, L. Ylianttila, D. Leszczynski (Helsinki, FIN)
- 12.15 **OC119 5-HT₂ blockage prevents psoralen+UVA (PUVA)-induced suppression of delayed hypersensitivity**
P. Wolf^{1,2}, D.X. Nghiem², J.P. Walterscheid², H.N. Ananthaswamy², S.E. Ullrich² (¹Graz, AUT; ²Houston TX, USA)
- 12.30 **OC120 Ultraviolet radiation-induced immunosuppression in humans is prevented by topical nicotinamide (vitamin B₃) and is greater in men than women**
C.R. Patterson, G.M. Halliday, R.Stc. Barnetson, D.L. Damian (Sydney, AUS)
- 12.45 **OC121 DX5+ NKT cells from UV-irradiated mice regulate tumor immunity by suppressing CLT activity**
N.M. Khaskhely, Y. Matsumura, Y. Miyahara, N. Kazimi, S.N. Byrne, S.E. Ullrich (Houston TX, USA)
- 13.00 **OC122 Molecular changes in skin induced by exposure to different doses of ultraviolet A radiation**
M.P.F. Stapelberg, R.B.H. Williams, S.N. Byrne, G.M. Halliday (Sydney, AUS)
- 13.15 **OC123 Photoimmunoprotection by UVA radiation is determined by UVA dose and is dependent on cutaneous cyclic guanosine monophosphate (cGMP)**
V.E. Reeve, M. Allanson (Sydney, AUS)

Clinical applications of PDT*Chair: S.G. Bown (London, GBR)*

- 10.00 **IL124** **Optical diagnosis and treatment in hollow organs**
L.B. Lovat (London, GBR)
- 10.30 **IL125** **Photodynamic therapy for cancers of the head and neck**
P.-J. Lou^{1,2}, C. Hopper², S.G. Bown² (¹Taipei, TRE; ²London, GBR)
- 11.00 **IL126** **Photodynamic diagnosis and photodynamic therapy for brain tumors**
H. Kostron (Innsbruck, AUT)
- 11.30 **IL127** **Review of interstitial photodynamic therapy for tumours of solid organs**
S.G. Bown (London, GBR)
- 12.00 **OC128** **Synergistic combination of photochemical treatment and bleomycin on tumor growth**
K. Berg, A. Dietze, O. Kaalhus, A. Høgset (Oslo, NOR)
- 12.15 **OC129** **Evaluation of the photosensitizer Tookad for photodynamic therapy on the Syrian golden hamster cheek pouch model: light dose, drug dose and drug-light interval effects**
F. Borle, A. Radu, P. Monnier, H. van den Bergh, G. Wagnières (Lausanne, SUI)
- 12.30 **OC130** **Photodynamic therapy and fluorescent diagnostics in head and neck cancer patients with different photosensitisers**
E.G. Vakulovskaya (Moscow, RUS)
- 12.45 **OC131** **Photodynamic applications of superficial bladder cancer: from detection to treatment!**
P. Jichlinski (Lausanne, SUI)
- 13.00 **PII49** **Photodynamic therapy for COPD treatment. Clinical results**
N.E. Vasiliev (Novosibirsk, RUS)
- 13.15 **PII54** **Ultraviolet-induced autofluorescence characterization of normal and tumoral esophageal epithelium cells**
S. Villette, C. Vever-Bizet, G. Bourg-Heckly (Paris, FRA)
- 13.30 **PII45** **Combined action of Visudyne and coherent or non-coherent light on melanoma cells**
P. Nowak-Sliwinska, G. Stochel, K. Urbanska (Cracow, POL)

Tuesday, September 6, Morning**Lamartine 3**

Light-regulation in plants: growth and rhythms*Chair: S. Braslavsky (Mülheim, GER)*

- 10.00 **IL133** **Light signalling and plant transcriptome patterns**
J.J. Casal (Buenos Aires, ARG)
- 10.30 **IL134** **Quantitative trait locus analysis of the phase of the *Arabidopsis* circadian clock**
C. Darrah¹, B. Taylor¹, K. Edwards², P. Brown², A. Hall³, H. McWatters¹ (¹Oxford, GBR; ²Edinburgh, GBR; ³Liverpool, GBR)
- 11.00 **IL135** **A GAF-domain mutation of phytochrome a impairs chromophore incorporation and light responses in *Arabidopsis***
J. Mateos¹, J.J. Casal¹, S.E. Braslavsky², W. Gärtner² (¹Buenos Aires, ARG; ²Mülheim, GER)
- 11.30 **PII97** **Isolation and characterization of a novel photomorphogenic and circadian clock mutant in *Arabidopsis***
É. Kevei¹, P. Gyula¹, R. Tóth¹, B. Fehér¹, A. Viczián¹, L. Kozma-Bognár^{1,2}, A.J. Millar², F. Nagy¹ (¹Szeged, HUN; ²Edinburgh, GBR)
- 11.45 **PII98** **New light signalling component affecting circadian clock in *Arabidopsis thaliana***
B. Fehér¹, É. Kevei¹, P. Gyula¹, R. Tóth¹, V. Sokolova¹, L. Kozma-Bognár^{1,2}, A.J. Millar², F. Nagy¹ (¹Szeged, HUN; ²Edinburgh, GBR)

Photoprotection and sunscreens*Chair: A.R. Young (London, GBR)*

- 14.30 **IL136** **Low-dose UVB induces a p53-dependent gene program that increases the resilience of keratinocytes against further UVB-insults**
D. Decraene¹, K. Smaers¹, D. Maes², M. Matsui², L. Declercq³, M. Garmyn¹ (¹Leuven, BEL; ²Melville NY, USA; ³Oevel, BEL)
- 15.00 **IL137** **Heat shock proteins in intrinsic photoprotection**
F. Trautinger (Vienna, AUT)
- 15.30 **IL138** **Biological effects of simulated ultraviolet daylight – New approach to investigate daily photoprotection**
S. Seité¹, C. Medaisko¹, C. Bredoux¹, F. Christiaens¹, D. Compan-Zaouati², H. Zucchi¹, D. Lombard¹, A. Fourtanier¹ (¹Clichy, FRA; ²Chevilly, FRA)
- 16.00 **IL139** **Effects of repeated sub-erythemal UVR exposure on human skin *in vivo* and the role of sunscreens in their prevention**
A.R. Young (London, GBR)
- 16.30 **OC140** **Cosmesis-relevant biophysical changes in Asian volunteers induced by extensive exposure to sub-erythemal doses of ultraviolet daylight. Influence of sun filters and textiles**
W. Baschong¹, U. Osterwalder¹, M. Schaumann¹, C. Artmann² (¹Basel, SUI; ²München, GER)
- 16.45 **OC141** **Sunscreen use related to UV exposure, age, sex and occupation based on personal dosimeter readings and sun behaviour diaries**
E. Thieden, P.A. Philipsen, J. Sandby-Møller, H.C. Wulf (Copenhagen, DEN)
- 17.00 **PI58** **Twelve-month topical study to determine the influence of bisoctrizole (Tinosorb® M- Active) on photocarcinogenesis in hairless mice**
D.B. Learn¹, C.P. Sambuco¹, P.D. Forbes¹, A.M. Hoberman¹, J.R. Plautz², U. Osterwalder² (¹Horsham PA, USA; ²Basel, SUI)
- 17.15 **PI71** **Pulsed radiation studies of natural UV filters extracted from lichens: possible sunscreens**
R. Edge (Keele, GBR)

Tuesday, September 6, Afternoon**Lamartine 2**

Photochemistry and phototoxicity of drugs*Chair: N. Gibbs (Manchester, GBR)*

- 14.30 **IL142** **An outbreak of photoallergy**
J. Ferguson (Dundee, GBR)
- 15.00 **IL143** **Drug-nucleoside interactions in the excited states: a model to investigate the basic mechanisms of drug-photosensitized DNA damage**
M.Á. Miranda (Valencia, ESP)
- 15.30 **IL144** **Spectrum of cross-photosensitization in patients with contact photoallergy to ketoprofen: associated photoallergies to non-benzophenone-containing molecules**
F. Aubin, P. Girardin, Ph. Humbert, R. Laurent, M. Vigan (Besançon, FRA)
- 16.00 **IL145** **Identification of photoallergic potential in drugs and other chemicals**
M.D. Barratt (Bedford, GBR)
- 16.30 **OC147** **Primary processes in the photochemistry of fluoroquinolones**
S. Navaratnam (Warrington, GBR)
- 16.45 **OC148** ***In vitro* phototoxicity of lovastatin and a selected group of vitamin D3 isomers**
P.D. Forbes, C.P. Sambuco, S.M. Ksenzenko, D.B. Learn, A.M. Hoberman (Horsham PA, USA)
- 17.00 **OC149** **Carbanion-mediated photocages: from drug photostability to carbanion kinetics and new photocages**
J.C. Scaiano, M. Lukeman, E. Gagnon, J. Blake (Ottawa, CAN)

Tuesday, September 6, Afternoon

Lamartine 3

Plant photobiology in extreme environments

Chair: T.V. Callaghan (Abisko, SWE)

- 14.30 **IL150** **Plants in cold environments: an introduction to the symposium**
T.V. Callaghan (Abisko, SWE)
- 15.00 **IL151** **Tracking ancient UV-B fluxes**
B. Lomax¹, D. Beerling¹, T. Callaghan^{1,2}, W. Fraser³, M. Harfoot⁴, J. Pyle⁴, S. Self³, M. Sephton³,
C. Wellman¹ (¹Sheffield, GBR; ²Abisko, SWE; ³Milton Keynes, GBR; ⁴Cambridge, GBR)
- 15.30 **IL152** **Can marine microorganisms influence the melting of the Arctic pack ice?**
C. Leck (Stockholm, SWE)
- 16.00 **IL153** **Cryophylic cyanobacteria and algae and their succession in deglaciated landscapes**
J. Elster¹, J. Svoboda², K. Kaštovská¹ (¹České Budějovice, CZE; ²Mississauga, CAN)

Tuesday, September 6, Afternoon

Theatre

- 17.30 **ESP General Assembly**

Tuesday, September 6, Evening

Victoria

- 20.00 **Banquet – ESP Medals Awards**

Wednesday, September 7, Morning

Theatre

- 08.45 **PL154 Photobiology Update**
Looking for novel circadian clock mutants in *Arabidopsis*
 É. Kevei¹, P. Gyula¹, B. Fehér¹, R. Tóth², V. Sokolova¹, S. Davis², L. Kozma-Bognár^{1,2}, A. Millar³,
 F. Nagy¹ (¹Szeged, HUN; ²Köln, GER; ³Edinburgh, GBR) *introduced by J. Bornman (Slagelse, DEN)*

09.30 *Coffee Break*

Wednesday, September 7, Morning

Lamartine 1**Ocular light damage***Chair: D.H. Sliney (Gunpowder MD, USA)*

- 10.00 **IL155 The geometry and spectral aspects of ocular exposure influences risks**
 D.H. Sliney (Gunpowder MD, USA)
- 10.30 **IL156 Light damage to the retina: model and reality**
 C.E. Remé (Zürich, SUI)
- 11.00 **IL157 The photoreactivity of retinal melanin and lipofuscin granules**
M.E. Boulton, M.B. Rozanowska (Cardiff, GBR)
- 11.30 **IL158 Optical radiation effects upon the lens**
P. Soderberg¹, S. Löfgren², M. Ayala³, X. Dong¹, V. Mody¹, M. Kakar¹, L Meyer¹ (¹Stockholm, SWE;
²Lincoln, NE, USA; ³Örebro, SWE)
- 12.00 **PI43 Winter eye protection for ultraviolet radiation**
D.H. Sliney¹, D. Sarkany² (¹Gunpowder MD, USA; ²Whistler, CAN)

Cellular response to solar UV*Chair: E. Sage (Paris, FRA)*

- 10.00 **IL159** **UVA modulation of iron and heme homeostasis – a crucial role for heme oxygenase**
R.M. Tyrrell, G. Edwards, C. Raval, S.A. Mitchell (Bath, GBR)
- 10.30 **IL160** **Role of cell cycle checkpoint proteins in UVA-induced S-phase arrest in mammalian cells**
P.-M. Girard, F. Delacôte, E. Sage (Orsay, FRA)
- 11.00 **IL161** **UV responses in NER-deficient mouse keratinocyte cultures**
G.J. Stout¹, J. de Wit², J.R. Mitchell², J.H.J. Hoeijmakers², F.R. de Gruijl¹, L.H.F. Mullenders¹,
C. Backendorf¹ (¹Leiden, NED; ²Rotterdam, NED)
- 11.30 **IL162** **Mono-ubiquitination of PCNA: a key modification for the polymerase switch after UV irradiation in human cells**
P. Kannouche (Villejuif, FRA)
- 12.00 **OC163** **RhoB up-regulation induced by UVB controls survival response of human keratinocytes**
B. Canguilhem, A. Pradines, C. Baudouin, C. Boby, I. Lajoie-Mazenc, M. Charveron, G. Favre (Toulouse, FRA)
- 12.15 **OC164** **Overexpression of phospholipid hydroperoxide glutathione peroxidase in human dermal fibroblasts abrogates UVA irradiation-induced expression of interstitial collagenase/matrix-metalloproteinase-1 by suppression of phosphatidylcholine hydroperoxide-mediated NFκB activation and interleukin-6 release**
S. Sulyok¹, J. Wenk², J. Schüller^{1,2}, C. Hinrichs^{1,2}, T. Syrovets¹, N. Azoitei¹, M. Podda³, T. Wirth¹, T. Simmet¹, K. Scharffetter-Kochanek^{1,2} (¹Ulm, GER; ²Cologne, GER; ³Frankfurt, GER)
- 12.30 **PI30** **UVB induced sunburn cell formation of human keratinocytes is mediated through the Ask-1-p38MAPK cascade**
A. Van Laethem, M. Garmyn, P. Agostinis (Leuven, BEL)

Wednesday, September 7, Morning**Lamartine 3**

Acclimation, stress and regulation of photosynthesis*Chair: E.-M. Aro (Turku, FIN), J.F. Bornman (Slagelse, DEN)*

- 10.00 **IL165** **Analysis of state transitions in *Chlamydomonas* and *Arabidopsis***
J.-D. Rochaix, S. Bellafiore, F. Barneche, L. Legendre, A. Willig, S. Miras (Geneva, SUI)
- 10.30 **IL166** **Elucidating the Mg-ProtoIX mediated signalling pathway**
Å. Strand (Umeå, SWE)
- 11.00 **IL167** **Carbon metabolite sensing and signalling: the role of the trehalose pathway**
M.J. Paul (Harpenden, GBR)
- 11.30 **IL168** **Chloroplast-mediated regulation of *Arabidopsis* transcriptome**
M. Piippo, Y. Allahverdiyeva, N. Battchikova, E.-M. Aro (Turku, FIN)
- 12.00 **OC169** **Singlet oxygen as a stress factor and signal in *Rhodobacter***
*J. Glaeser*¹, *M. Zobawa*², *F. Lottspeich*², *G. Klug*¹ (¹Giessen, GER; ²Martinsried, GER)
- 12.15 **OC170** **Molecular events underlying excitation energy control in the Photosystem II antenna**
A.V. Ruban (Sheffield, GBR)

Animal models of photocarcinogenesis*Chair: D. Mitchell (Smithville TX, USA), F.R. de Gruijl (Leiden, NED)*

- 15.30 **IL171** **Regulation of DNA repair in E2F1 transgenic and knockout mice**
D.L. Mitchell, T. Berton, R. Guo, L. Paniker, D. Johnson (Smithville TX, USA)
- 16.00 **IL172** **DNA lesions and mutations induced by ultraviolet A and B radiation in human and mouse cells**
G.P. Pfeifer, A. Besaratinia (Duarte CA, USA)
- 16.30 **IL173** **Induction of CPD retaining basal cells after solar simulating UV-irradiation of human skin**
B. Volkmer¹, S. Henning¹, D. Mitchell², E.W. Breitbart¹, R. Greinert¹ (¹Buxtehude, GER; ²Smithville TX, USA)
- 17.00 **IL174** **Oncogenes, signal transduction and melanoma in *Xiphophorus***
S. Meierjohann¹, C. Wellbrock², A. Gómez³, E. Geissinger¹, C. Froschauer¹, E. Wende¹, M. Papp¹, A. Kraiss¹, M. Schartl¹ (¹Würzburg, GER; ²London, GBR; ³Ribera de Cabanes, ESP)
- 17.30 **IL175** **Role of cell cycle regulating genes in susceptibility to UV-induced melanomas in *Xiphophorus* hybrids**
R.S. Nairn, R. Beard, D. Trono, A.P. Butler (Smithville TX, USA)
- 18.00 **OC176** **Deficient UV induction of melanoma in HGF/SF transgenic recessive yellow (*Mc1r^{el/e}*) mice**
F.P. Noonan¹, G. Merlino², A. Wolnicka-Glubisz¹, M. Anver³, E. De Fabo¹ (¹Washington DC, USA; ²Bethesda MD, USA; ³Frederick MD, USA)
- 18.15 **PI42** **Quantitative and temporal differences in UVR dose-dependent skin responses in hairless rodents (mice, rats, and guinea pigs)**
C.P. Sambuco, P.D. Forbes, D.B. Learn, M. Arocena, A.M. Hoberman (Horsham PA, USA)

Wednesday, September 7, Afternoon**Lamartine 2**

Antimicrobial PDT*Chair: G. Jori (Padova, ITA)*

- 15.30 **IL177** **Photodynamic therapy-based inactivation of viruses in the presence of red blood cells**
P. Ziolkowski¹, M.A. Valles², M.A. de Madariaga², O.I. Koifman³, R. Bonnett⁴ (¹Wroclaw, POL; ²Barcelona, ESP; ³Ivanovo, RUS; ⁴London, GBR)
- 16.00 **IL178** **Antimicrobial PDT with phenothiazinium dyes: new mechanistic findings**
M. Hamblin, T. Ndemidova, G. Ptegos (Boston MA, USA)
- 16.30 **IL179** **Photodynamic therapy of bacterial and fungal infections with phthalocyanines: basic and pre-clinical studies**
G. Jori¹, G. Roncucci² (¹Padova, ITA; ²Firenze, ITA)
- 17.00 **IL180** **Antimicrobial PDT: we have heard the theory, what about the practice?**
S.B. Brown (Leeds, GBR)
- 17.30 **OC181** **Photosensitization-based inactivation of harmful mycomycetes**
Ž. Lukšienė, D. Pečiulytė, A. Lugauskas (Vilnius, LTU)
- 17.45 **OC182** **Porphyrin polyamine conjugates : a new strategy for antimicrobial chemotherapy**
V. Sol, P. Branland, F. Lamarche, G. Garcia, R. Granet , B. Verneuil, P. Krausz (Limoges, FRA)
- 18.00 **OC183** **Photodynamic killing of *Leishmania major* with cationic dyes**
O.E. Akilov¹, S. Kosaka¹, J.W. Foley², T. Hasan¹ (¹Boston MA, USA; ²Cambridge MA, USA)
- 18.15 **PII70** **The treatment of leishmaniasis using photodynamic therapy**
C. Bristow, R.W. Boyle, T. Paget (Hull, GBR)

Spectroscopic studies of fast and ultrafast events in photobiology*Chair: M.M. Martin (Paris, FRA)*

- 15.30 **IL184** **Ultrafast electron transfer in photosynthetic reaction centres: on the way to a molecular understanding of photosynthetic electron transfer**
W. Zinth (München, GER)
- 16.00 **IL185** **Ultrafast dynamics in the Green Fluorescent Protein (GFP) and the GFP chromophore**
D. Stoner-Ma¹, A.A. Jaye², P. Tonge¹, S.R. Meech² (¹Stony Brook NY, USA; ²Norwich, GBR)
- 16.30 **IL186** **Ultrafast photo-isomerization of protonated retinal Schiff bases in different environments**
S. Schenk¹, G. Zgrablic¹, F. van Mourik¹, S. Haacke^{1,2}, M. Chergui¹ (¹Lausanne, SUI; ²Strasbourg, FRA)
- 17.00 **IL187** **Fast molecular processes in *Blepharisma japonicum*'s light perception**
M. Mahet¹, P. Plaza¹, M.M. Martin¹, G. Checcucci², F. Lenci² (¹Paris, FRA; ²Pisa, ITA)
- 17.30 **OC188** **Electronic structure, spectroscopy, ultrafast dynamics and aerobic reactivity of trichochromes: natural components of pheomelanins**
J.D. Simon (Durham NC, USA)
- 17.45 **PII86** **Ultrafast events in the Photoactive Yellow Protein chromophore : protein vs solution environment**
A. Espagne¹, P. Changenet-Barret¹, P. Plaza¹, K.J. Hellingwerf², M.M. Martin¹ (¹Paris, FRA; ²Amsterdam, NED)
- 18.00 **OC189** **Structural characterisation of the fucoxanthin chlorophyll-a/c protein complexes using resonance Raman spectroscopy: the effect on light harvesting and energy transfer dynamics**
L. Premvardhan¹, L. Bordes¹, H. Fey², C. Buchel², B. Robert¹ (¹Gif-sur-Yvette, FRA; ²Frankfurt, GER)
- 18.15 **OC190** **Observation of sub-100 ps conformational changes in photolyzed carbonmonoxy-myoglobin probed by time-resolved circular dichroism**
T. Dartigalongue, F. Hache (Palaiseau, FRA)

Thursday, September 8, Morning

Theatre

08.45 **PL191 Photobiology Update**
Photoprotection and photorestitution of cultural heritage
 R. Salimbeni (Firenze, ITA) *introduced by F. Ghetti (Pisa, ITA)*

09.30 *Coffee Break*

Thursday, September 8, Morning

Lamartine 1**Mechanisms of plant UV-response***Chair: R. Ulm (Freiburg, GER)*

10.00 **IL192 Regulation of UVB-induced photomorphogenesis in *Arabidopsis***
 A. Baumann¹, A. Oravec¹, Z. Mate², F. Nagy², R. Ulm¹ (¹Freiburg, GER; ²Szeged, HUN)

10.30 **IL193 UV-B signalling and protection in *Arabidopsis***
 G.I. Jenkins (Glasgow, GBR)

11.00 **IL194 Impact of UV-B on maize**
V. Walbot¹, P. Casati² (¹Stanford CA, USA; ²Rosario, ARG)

11.30 **IL195 MAP kinases at the crossroads of UV-B and biotic stress signalling pathways**
 J. Stratmann (Columbia SC, USA)

12.00 **PII99 Analysis of the maize leaf proteome after various UV-B treatments of lines differing in UV-B sensitivity**
P. Casati^{1,2}, X. Zhang³, A.L. Burlingame³, V. Walbot¹ (¹Stanford CA, USA; ²Rosario, ARG; ³San Francisco CA, USA)

12.15 **OC196 The role of the FtsH and DegP proteases in the repair of Photosystem II after damage by UV-B radiation in *Synechocystis***
I. Vass¹, O. Cheregi¹, C. Sicora¹, P.B. Kos¹, P.J. Nixon² (¹Szeged, HUN; ²London, GBR)

PDT: oral communications*Chair: J. Piette (Liège, BEL)*

- 10.00 **OC197** **Formulation of a thermosetting hydrogel of hexylaminolevulinat targeting Barrett's oesophagus**
S. Collaud¹, F. Evangelisti¹, L. James¹, Q. Peng², R. Gurny¹, N. Lange¹ (¹Geneva, SUI; ²Oslo, NOR)
- 10.15 **OC198** **On the importance of light delivery and intensity measurements during ALA PDT in otorhinolaryngology**
T. Vidóczy, P. Baranyai, L. Csokonai Vitéz, B. Horváth (Budapest, HUN)
- 10.30 **OC199** **ALA-PDT effect on adhesion and cytoskeleton of the cultured human carcinoma and glioma cells**
A. Uzdensky^{1,2}, E. Kolpakova¹, A. Juzeniene¹, J. Moan¹ (¹Oslo, NOR; ²Rostov-on-Don, RUS)
- 10.45 **OC200** **ALA-induced endogenous porphyrins in arthritic rabbit knee tissues: a spectroscopic study**
S. Bagdonas, G. Kirdaitė, R. Rotomskis (Vilnius, LTU)
- 11.00 **OC201** **Meta-tetra(hydroxyphenyl)chlorin (mTHPC) aggregation state and interaction with plasma albumin**
S. Sasnouski^{1,2}, V. Zorin², I. Khludeev², F. Guillemin¹, L. Bolotine¹ (¹Vandoeuvre-les-Nancy, FRA; ²Minsk, BLR)
- 11.15 **PII23** **Time dependent subcellular localisation of mTHPC and apoptotic response in photosensitized MCF-7 cells**
A. François, S. Marchal, F. Guillemin, L. Bolotine (Vandoeuvre-Lès-Nancy, FRA)
- 11.30 **OC202** **The effect of photofrin-photodynamic therapy (PDT) on mechanisms of cell death in EMT6 murine cells**
S.L. Hankin, T.J. Stephenson, M.W.R. Reed, A.G. Pockley, N.J. Brown (Sheffield, GBR)
- 11.45 **OC203** **Effect of hypericin on intracellular localization of PKC in U-87 MG human glioma cells: competitive binding of Hyp and PMA to C1B sub-domain of PKC**
S. Kocanova¹, D. Jancura¹, T. Hornakova¹, A. Mateasik², D. Chorvat Jr.², J. Ulicny¹, J. Hritz¹, M. Refregiers³, J.C. Maurizot³, P. Miskovsky^{1,2} (¹Kosice, SVK; ²Bratislava, SVK; ³Orléans, FRA)
- 12.00 **OC204** **Enhanced selectivity of chemo and photochemotherapeutic agents via protease mediated drug delivery**
E. Dickson, R. Goyan, J. Kennedy, K. Latulippe, R. Pottier, J. Wojtyk (Kingston, CAN)
- 12.15 **OC205** **FLIM and SLIM for molecular imaging in PDT**
A. Rück, F. Dolp, C. Hülshoff, C. Hauser, C. Scalfi-Happ (Ulm, GER)
- 12.30 **OC206** **Analyses of monolayer cells by MALDI-TOF/MS - Applications for photodynamic therapy**
B. Maunit¹, M. Dodeller¹, N. Lourette¹, L. Bezdetsnaya², F. Guillemin², J.-F. Muller¹ (¹Metz, FRA; ²Nancy, FRA)
- 12.45 **PII42** **Development of sensitizers based on squaraine moiety for photodynamic therapy**
D. Ramaiah¹, K.T. Arun¹, K. Jyothish¹, B. Epe² (¹Trivandrum, IND; ²Mainz, GER)
- 13.00 **OC146** **Photochemical internalisation of EGFR-targeting toxin conjugates**
A. Weyergang, P.K. Selbo, W.L. Yip, K. Berg (Oslo, NOR)

Thursday, September 8, Morning

Lamartine 3

Photochemistry and photobiology of biomolecules

Chair: J. Cadet (Grenoble, FRA)

- 10.00 **OC207 Interaction of UVC/UVB radiation with DNA simple and double helices: from photon absorption to photodamage**
D. Markovitsi¹, E. Emanuela¹, T. Gustavsson¹, E. Lazzarotto¹, R. Lavery², S. Marguet¹, P. Millié¹, D. Onidas¹, A. Sharonov¹, F. Talbot¹, K. Zakrzewska² (¹Gif-sur-Yvette, FRA; ²Paris, FRA)
- 10.15 **OC208 Photocaged radicals for the studies of oxidative DNA damage**
Y. Wang (Riverside CA, USA)
- 10.30 **OC209 Application of UV laser photochemistry to the studies of histone-DNA interactions and transcription factor binding to nucleosomes**
D. Angelov^{1,2}, H. Menoni^{1,2}, J. Cadet³, P. Bouvet¹, S. Dimitrov^{1,4} (¹Lyon, FRA; ²Sofia, BUL; ³Grenoble, FRA; ⁴La Tronche, FRA)
- 10.45 **OC210 Lipid hydroperoxides as an endogenous precursor of singlet molecular oxygen in the presence of metal ions and other reactive species**
S. Miyamoto¹, G.R. Martinez^{1,2}, A.P. Bortoletto Martins¹, M.H. Gennari Medeiros¹, P. Di Mascio¹ (¹São Paulo, BRA. ²Curitiba, BRA)
- 11.00 **OC211 Singlet oxygen-mediated formation of protein peroxides in cells and its consequences**
V.V. Agon, M. Gracanin, P.E. Morgan, M.J. Davies (Sydney, AUS)
- 11.15 **OC212 UVA radiation is highly mutagenic in cells that are unable to repair 7,8-dihydro-8-oxoguanine in *Saccharomyces cerevisiae***
E. Sage¹, S. Kozmin¹, G. Slezak², A. Reynaud-Angelin¹, S. Boiteux² (¹Orsay, FRA; ²Fontenay aux Roses, FRA)
- 11.30 **OC213 Sensitivity to polychromatic UV-radiation of strains of *Deinococcus radiodurans* differing in their DNA repair capacity**
P. Rettberg¹, U. Pogoda de la Vega¹, T. Douki², J. Cadet² (¹Köln, GER; ²Grenoble, FRA)
- 11.45 **OC214 Killed But Metabolically Active (KBMA) microbes: a new vaccine paradigm for eliciting effector T cell responses and protective immunity**
D.G. Brockstedt¹, K.S. Bahjat¹, M.A. Giedlin¹, W. Liu¹, M. Leong¹, W. Lockett¹, Y. Gao¹, P. Schnupf³, D. Kapadia¹, G. Castro¹, J.Y.H. Lim¹, A. Sampson-Johannes¹, A.A. Herskovits³, A. Stassinopoulos¹, H.G. Archie Bower², J.E. Hearst¹, D.A. Portnoy³, D.N. Cook¹, T.W. Dubensky Jr.¹ (¹Concord CA, USA; ²Portland OR, USA; ³Berkeley CA, USA)
- 12.00 **OC215 Experimental and theoretical study of the interaction of single-stranded DNA homopolymers and a monomethine cyanine dye: nature of specific binding**
L. Mikelsons, C. Carra, M. Shaw, C. Schweitzer, J.C. Scaiano (Ottawa, CAN)
- 12.15 **OC216 Photoinduced DNA damage by quinolizinium derivatives – Singlet oxygen generation and unusual formation of hydroxyl radicals**
H. Ihmels¹, C. Bohne², K. Faulhaber³, B. Giese⁴, A. Hofmann³, A.-K. Köhler⁴, A. Salbach¹, M.A.L. Sheepwash² (¹Siegen, GER; ²Victoria, CAN; ³Würzburg, GER; ⁴Basel, SUI)
- 12.30 **OC217 Photoprocesses in densely packed stable stacking aggregates of nucleic bases of candidates for the role of first genetic matrixes**
V.L. Rapoport, V.M. Malkin, S.V. Zorina, S.M. Komarov, V.V. Goriuchko (St. Petersburg, RUS)

Monday, September 5, (14.00 – 15.30)
Poster session I**Raphaël**

- PI1** **Two diastereomeric (6-4) adducts from photoreaction of uracil in frozen aqueous solution**
M.D. Shetlar, V.J. Basus (San Francisco CA, USA)
- PI2** **Time-resolved study of thymine dimer formation**
S. Marguet, D. Markovitsi (Gif-sur-Yvette, FRA)
- PI3** **Photo-induced guanine radical cation in short oligonucleotide is able to cross-link with lysines containing peptide**
S. Perrier¹, J. Hau², D. Gasparutto¹, J. Cadet¹, A. Favier¹, J.-L. Ravanat¹ (¹Grenoble, FRA; ²Lausanne, SUI)
- PI4** **Crystal structure of the d(TpA) thymine-adenine photoadduct**
R.J.H. Davies, J.F. Malone (Belfast, GBR)
- PI5** **Antigenotoxic activity of two essential oils (*Origanum compactum* and *Cinnamomum camphora*) against UV and 8-MOP+UVA induced mutagenic and recombinogenic events in diploid yeast (*Saccharomyces cerevisiae*)**
F. Bakkali^{1,2}, S. Averbeck², D. Averbeck², M. Idaomar¹ (¹Tétouan, MAR; ²Orsay, FRA)
- PI6** **Photochemistry of DNA in spores of *Bacillus subtilis***
T. Douki¹, B. Setlow², P. Setlow² (¹Grenoble, FRA; ²Farmington CT, USA)
- PI7** **Quantification of 4-hydroxy-2-nonenal adduct to glutathione and to DNA in humans cells**
O. Falletti, J. Cadet, A. Favier, T. Douki (Grenoble, FRA)
- PI8** **Study of the photobiological effects of 8-methoxypsoralen (8-MOP) and UVA in eukaryotic cells using DNA microarray technology**
M. Dardalhon¹, W. Lin², A. Nicolas², D. Averbeck¹ (¹Orsay, FRA; ²Paris, FRA)
- PI9** **Comparison between 8-oxo-7,8-dihydro-2'-deoxyguanosine and 1,N2-etheno-2'-deoxyguanosine levels in lung DNA of rats exposed to cigarette smoke**
S.A. Marques, A.P.M. Loureiro, O.F. Gomes, C.C. Garcia, P. Di Mascio, M.H.G. Medeiros (São Paulo, BRA)
- PI10** **Reaction of singlet oxygen with 2'-deoxyguanosine and 8-methoxy-2'-deoxyguanosine**
G.R. Martinez¹, J.-L. Ravanat², J. Cadet², M.H.G. Medeiros³, P. Di Mascio³ (¹Curitiba, BRA; ²Grenoble, FRA; ³São Paulo, BRA)
- PI11** **Steady-state and time-resolved studies on ketoprofen-thymine dyads**
C. Paris, N. Belmadoui, M.J. Climent, S. Encinas, M.Á. Miranda (Valencia, ESP)
- PI12** **Photoinduced binding of ruthenium trisbipyrazine on 8-oxoguanine and inhibition of the replicative T4 polymerase**
N. El-Akra, J-P. Souchard, P. Vicendo (Toulouse, FRA)
- PI13** **Photostability of ruthenium complexes in presence of proteins: influence in DNA photosensitization**
L. Bijeire, P. Vicendo (Toulouse, FRA)
- PI14** **Spore photoproduct lyase - characterization of an iron-sulfur DNA repair enzyme**
O. Berteau, S. Ollagnier-de-Choudens, T. Douki, M. Atta, M. Fontecave (Grenoble, FRA)
- PI15** **DNA-DNA crosslinking at guanines: a new and major class of oxidatively generated damage by UV laser biphotonic ionization in DNA**
H. Menoni^{1,2}, D. Gasparutto³, J.-L. Ravanat³, J. Cadet³, S. Dimitrov⁴, D. Angelov^{1,2} (¹Lyon, FRA; ²Sofia, BUL; ³Grenoble, FRA; ⁴La Tronche, FRA)
- PI16** **Ozone induces singlet molecular oxygen generation in the presence of DNA**
S. Miyamoto¹, G.R. Martinez^{1,2}, M.H.G. Medeiros¹, P. Di Mascio¹ (¹São Paulo, BRA, ²Paraná, BRA)

- PI17 Interplay of nitration and oxidation reactions in DNA: insights from laser flash photolysis and oxygen-18 labeling experiments**
R. Misiaszek, C. Crean, N.E. Geacintov, V. Shafirovich (New York NY, USA)
- PI18 Miniaturized instrumentation for the high throughput screening of DNA repair inhibitors**
R. Hara¹, A. North¹, J.E. Hearst^{1,2}, S. Yeung², K. Singh², R.A. Mathies² (¹Concord CA, USA; ²Berkeley CA, USA)
- PI19 Effect of (5'S)-5',8-Cyclo-2'-deoxyadenosine on the conformation of di and trinucleotides**
B. Karwowski, J. Gaillard, A. Grand, J.Cadet (Grenoble, FRA)
- PI20 Study of 8-bromo-2'-deoxyinosine by photochemical and radiolytic methods**
L. Jimenez^{1,2}, S. Encinas¹, M.Á. Miranda¹, M. Russo², M. D'Angelantonio², Q.G. Mulazzani², C. Chatgialiloglu² (¹Valencia, ESP; ²Bologna, ITA)
- PI21 A plasmid biochip for DNA repair measurement, adaptation of HeLa cells to UVB irradiation**
J.-F. Millau, A. Micoud, S. Caillat, A. Favier, S. Sauvaigo (Grenoble, FRA)
- PI22 Formation and biological significance of one-electron oxidation products of 5-hydroxypyrimidine residues**
Ph. Simon¹, D. Gasparutto¹, C. Saint-Pierre¹, J.R. Wagner², A. Favier¹, J. Cadet¹ (¹Grenoble, FRA; ²Sherbrooke, CAN)
- PI23 Modification of the genotoxic effect of UV-irradiation by chemicals**
G. Emri¹, C. Bayerl², E. Remenyik¹, I. Horkay¹ (¹Debrecen, HUN; ²Mannheim, GER)
- PI24 Studies on the photocleavage of nucleic acids by the dibenzo[b,g]quinolizinium salt and its dimer**
D. Otto¹, A. Bergen¹, H. Ihmels¹, G. Viola² (¹Siegen, GER; ²Padova, ITA)
- PI25 Long-time fluorescence components of poly(dA).poly(dT)**
F. Talbot, D. Markovitsi, T. Gustavsson, E. Lazzarotto (Gif-sur-Yvette, FRA)
- PI26 A comparison of solar UV induced DNA-damaging effects between Southern and Central Europe and Arctic high latitudes**
A. Bérces¹, S.A. Chernouss², H. Lammer³, N.K. Belisheva², G. Kovács¹, H.I.M. Lichtenegger³, G. Rontó¹ (¹Budapest, HUN; ²Apatity, RUS; ³Graz, AUT)
- PI27 Ground-based measurement of solar light intensity (illuminance), temperature, and relative humidity from the tropics (Turen, East Java, Indonesia) between 1995 and 2001**
L. Fidhiany, K. Winckler (Berlin, GER)
- PI28 Effects of sublethal (7.4 W/m²) UVA irradiation on activity levels of oxidative defense enzymes (catalases, superoxide dismutases, glutathione reductase) and on protein oxidation and survival after lethal UVA in *Escherichia coli***
J.D. Hoerter, A.A. Arnold, A. Shibuya, D. Kuczynska, C.S. Ward, M.G. Sauer, A. Gizachew, T.M. Hotchkiss, T. Fleming, S. Johnson (Big Rapids MI, USA)
- PI29 The role of Bach-1 and NF-E2-related factor 2 in ultraviolet A (oxidant) mediated activation of heme oxygenase-1 in human skin cells**
G.P. Edwards, C.M. Raval, S.A. Mitchell, R.M. Tyrrell (Bath, GBR)
- PI30 UVB induced sunburn cell formation of human keratinocytes is mediated through the Ask-1-p38MAPK cascade**
A. Van Laethem, M. Garmyn, P. Agostinis (Leuven, BEL)
- PI31 Efficacy of different UV emitting sources in the induction of T cell apoptosis**
Z. Novák¹, A. Bérces², G. Rontó², A. Dobozy¹, L. Kemény¹ (¹Szeged, HUN; ²Budapest, HUN)
- PI32 Induction of CCL21/SLC and dendritic cell activation by photodynamic therapy**
S.O. Gollnick, B. Owczarczak, B.W. Henderson (Buffalo NY, USA)
- PI33 Induction of tolerance by UV-induced, platelet activating factor-stimulated, IL-10 secreting, B cells**
Y. Matsumura, D.X. Nghiem, Y. Miyahara, S.N. Byrne, S.E. Ullrich (Houston TX, USA)

- PI34 The effects of TL01 phototherapy on UVB-induced Langerhans' cell, CD11b+ and iC3b+ cell trafficking in polymorphic light eruption (PLE)**
A. Blackburn, S. Winhoven, M. Brownrigg, L.E. Rhodes, N.K. Gibbs (Manchester, GBR)
- PI35 Directing the immune system by light**
C.H. Self, M.-C. Fawcett, A. Self, J.A. Smith, S. Thompson (Newcastle-upon-Tyne, GBR)
- PI36 Modulation of contact hypersensitivity in mice by photolysis products of psoralens**
A.Ya. Potapenko, L.A. Kozir, Z.I. Moshnina, A.A. Kyagova (Moscow, RUS)
- PI37 Lack of antigen-specific immunity in the very early stage of photocarcinogenesis in an animal model**
É. Remenyik^{1,2}, N.M. Wikonkál^{1,2,3}, W. Zhang¹, V. Paliwal¹, D.E. Brash¹ (¹New Haven CT, USA; ²Debrecen, HUN; ³Budapest, HUN)
- PI38 Effect of UV irradiation on cellular responses and DNA damage of human keratinocytes harboring HPV16**
S. Mouret¹, T. Douki², S. Courdavault², A. Favier², J.C. Beani¹, M.T. Leccia¹ (¹La Tronche, FRA; ²Grenoble, FRA)
- PI39 The UV(B) fingerprint dominates the PTCH mutation spectrum of psoralen plus UVA-associated basal cell carcinomas**
E. Heitzer, A. Lassacher, H. Kerl, P. Wolf (Graz, AUT)
- PI40 Activating c-Kit exon 11 mutation is absent in Merkel cell carcinoma**
A. Lassacher, E. Heitzer, H. Kerl, P. Wolf (Graz, AUT)
- PI41 UVA sensitivity in Smith-Lemli-Opitz syndrome: possible involvement of cholesta-5,7,9(11)-trien-3 β -ol**
C.F. Chignell, B.M. Kukielczak, P.J. Bilski, Y.-Y. He, R.H. Sik (Research Triangle Park NC, USA)
- PI42 Quantitative and temporal differences in UVR dose-dependent skin responses in hairless rodents (mice, rats, and guinea pigs)**
C.P. Sambuco, P.D. Forbes, D.B. Learn, M. Arocena, A.M. Hoberman (Horsham PA, USA)
- PI43 Winter eye protection for ultraviolet radiation**
D.H. Sliney¹, D. Sarkany² (¹Gunpowder MD, USA; ²Whistler, CAN)
- PI44 UV-A irradiance at typical indoor life space**
S. Takeshita, M. Sasaki (Kanagawa, JPN)
- PI45 Efficacy of RGD-porphyrins on murine tumoral melanocytes: preliminary results**
A. Sparsa, K. Faucher, V. Sol, P. Krausz, M. Guilloton, F. Sturtz, C. Bédane (Limoges, FRA)
- PI46 Photodegradation of folic acid during extracorporeal photochemotherapy**
M. Der-Petrossian, M. Födinger, R. Knobler, F. Trautinger (Vienna, AUT)
- PI47 Efficacy of variable pulsed light in the treatment of hypertrichosis in 103 patients**
R. Knobler, H. Nahavandi, R. Neumann (Vienna, AUT)
- PI48 Impact of polymorphic light eruption on quality of life across four seasons and at a range of latitudes in Europe**
T.C. Ling¹, H.I. Richards¹, A.S. Janssens², F. Aubin³, C. Jansen⁴, L. Anastassopoulou⁵, A.J. Stratigos⁵, C. Antoniou⁵, T.I. Diepgen⁶, N.K. Gibbs¹, L.E. Rhodes¹ (¹Manchester, GBR; ²Leiden, NED; ³Besançon, FRA; ⁴Turku, FIN; ⁵Athens, GRE; ⁶Heidelberg, GER)
- PI49 The effects of TL-01 phototherapy on erythema and provocation responses in polymorphic light eruption (PLE)**
S. Winhoven, A. Blackburn, M. Brownrigg, L.E. Rhodes, N.K. Gibbs (Manchester, GBR)
- PI50 Acute UV irradiation and heat shock induced exon 26A mRNA and protein expression of elastin in human skin *in vivo***
J.Y. Seo, Z. Chen, S.R. Lee, Y.K. Kim, J.H. Chung (Seoul, KOR)

- PI51 Photochemical properties of aged human RPE melanosomes**
G. Szewczyk¹, A. Pawlak¹, M. Zareba¹, J.M. Burke², C.M.B. Skumatz², M.B. Rozanowska³, M.E. Boulton³, T. Sarna¹ (¹Krakow, POL; ²Milwaukee WI, USA; ³Cardiff, GBR)
- PI52 Modulation of oxidative damage, MAPK activation and cell death in neuronal cells by the fermented papaya preparation**
O.I. Aruoma¹, R. Colognato², K. Koike³, I. Laurenza², L. Benzi², L. Migliore² (¹London, GBR; ²Pisa, ITA; ³Gifu, JPN)
- PI53 Effect of ageing on the expression of cell defence genes after UVA irradiation of human male cutaneous fibroblasts using cDNA arrays**
F. Hazane¹, K. Valenti², S. Sauvaigo², A. Peinnequin¹, C. Mouret¹, A. Favier², J.-C. Beani^{1,2} (¹La Tronche, FRA; ²Grenoble, FRA)
- PI54 Can broad-spectrum sunscreens prevent the secretion of proinflammatory cytokines in human keratinocytes when exposed to the phototoxic drug lomefloxacin and UVA radiation?**
P. Reinhardt, M. Cybulski, S.M. Miller, C.L. Ferrarotto, R. Wilkins, Y. Deslauriers (Ottawa, CAN)
- PI55 Season of diagnosis is a strong prognostic factor in cancer. A possible role of sun-induced vitamin D**
A.C. Porojnicu, T.E. Robsahm, J. Moan (Oslo, NOR)
- PI56 Reaction of antioxidants with a stable colored free radical as a mean to assess their activity**
O. Friaa, D. Brault (Evry, FRA)
- PI57 Twelve-month topical study to determine the influence of bemotrizinol (Tinosorb® S) on photocarcinogenesis in hairless mice**
D.B. Learn¹, C.P. Sambuco¹, P.D. Forbes¹, A.M. Hoberman¹, J.R. Plautz², U. Osterwalder² (¹Horsham PA, USA; ²Basel, SUI)
- PI58 Twelve-month topical study to determine the influence of bisoctrizole (Tinosorb® M- Active) on photocarcinogenesis in hairless mice**
D.B. Learn¹, C.P. Sambuco¹, P.D. Forbes¹, A.M. Hoberman¹, J.R. Plautz², U. Osterwalder² (¹Horsham PA, USA; ²Basel, SUI)
- PI59 Is it possible to use the photodynamic effect to determine an antioxidant activity?**
M. Bancirova (Olomouc, CZE)
- PI60 Photoinduced DNA cleavage and binding studies of benzophenone-based sunscreen absorbers**
J. Kasavel, A.S. Sewlall, B.S. Martincigh (Durban, RSA)
- PI61 The effect of antioxidants on the *para*-aminobenzoic acid photosensitised formation of singlet oxygen**
A.M. Salim, B.S. Martincigh (Durban, RSA)
- PI62 An investigation of the photostabilisation of sunscreen absorbers by plant polyphenols**
G.J. Mturi, B.S. Martincigh (Durban, RSA)
- PI63 Incorporation of sunscreen-active agents in the nanospaces of layered double hydroxides**
M.K. Rotich, B.S. Martincigh (Durban, RSA)
- PI64 Photostability and photochemical analysis of commercial sunscreens**
T. Bunhu, B.S. Martincigh (Durban, RSA)
- PI65 Computational studies of the photodimerisation of 2-ethylhexyl- *para*-methoxycinnamate**
W. Waudu, H.G. Kruger, B.S. Martincigh (Durban, RSA)
- PI66 The UVB filter, 2-phenylbenzimidazole-5 sulfonic acid, enhances the formation of cyclobutane pyrimidine dimers and oxidative damage following UVA and simulated sunlight irradiation**
N. Bastien¹, M. Rouabhia², R. Drouin¹ (¹Sherbrooke, CAN; ²Quebec City, CAN)
- PI67 On the influence of application amount of sun protection products on their efficacy and photostability**
H. Tronnier¹, B. Garbe¹, U. Heinrich¹, D.Kockott² (¹Witten, GER; ²Hanau, GER)

- PI68 Influence of substitution at the benzylic position on the behavior of stereoisomeric phosphorus compounds as precursors of possible antioxidants**
 J. Pérez-Prieto¹, R.E. Galian¹, M.C. Morant-Miñana¹, P.O. Burgos², M.Á. Miranda¹, F. López-Ortiz²
 (¹Valencia, ESP; ²Almería, ESP)
- PI69 Antioxidant activity of mycosporine-like amino acids (MAAs) from marine dinoflagellates**
 M.R. Flores^{1,2,3}, M.I. Isla^{1,3}, M.E. Farías^{1,3}, E.W. Helbling^{2,3} (¹San Miguel de Tucumán, ARG; ²Playa Unión, ARG; ³CONICET, ARG)
- PI70 Seasonal changes in UV exposure**
S. Wengraitis, D. Sliney (Gunpowder MD, USA)
- PI71 Pulsed radiation studies of natural UV filters extracted from lichens: possible sunscreens**
 R. Edge (Keele, GBR)
- PI72 Betamethasone phototoxicity: *in vitro*, *in vivo* and *ex vivo* studies**
 G. Miolo¹, F. Gallochio¹, S. Caffieri¹, F. Baccichetti¹, C. Marzano¹, M.G. Zanirato²,
 G. Beyersbergen van Henegouwen¹ (¹Padova, ITA; ²Conselve, ITA)
- PI73 Effects of aqueous preparations on the phototoxicity of curcumin**
E.M. Bruzell¹, E. Morisbak¹, H.H. Tønnesen² (¹Haslum, NOR; ²Oslo, NOR)
- PI74 Singlet excited state properties of fluoroquinolones: emission of norfloxacin and derivatives in aqueous media**
 M.C. Cuquerella, M.Á. Miranda, F. Boscá (Valencia, ESP)
- PI75 Photophysical techniques for the study of drug-protein interactions: flurbiprofen-human serum albumin as model system**
M.C. Jiménez, I. Vayá, M.Á. Miranda (Valencia, ESP)
- PI76 Stereodifferentiation in the interaction between chiral carprofen and human serum albumin: from whole protein to model dyads**
V. Lhiaubet-Vallet, F. Boscá, M.Á. Miranda (Valencia, ESP)
- PI77 Singlet oxygen-mediated photodegradation of folic acid and photosensitizing activity of its photoproducts**
P. Vorobey^{1,2}, M.K. Off¹, A. Vorobey^{1,2}, J. Moan¹ (¹Oslo, NOR; ²Minsk, BLR)
- PI78 Effects of buffers, pH, and hydroxylated molecules on fluorescence emission of protein**
A.M. Almehdi, G.A. Ahmed (Al-Ain, UAE)
- PI79 Photosensitizing activity of di- or tetraaryl-porphyrins on HCT116 cells**
S. Banfi, E. Caruso, L. Buccafurni, R. Murano, E. Monti, M.B. Gariboldi, R. Ravizza, F. Terni (Varese, ITA)
- PI80 *In vitro* photodynamic efficacy of emodine bearing porphyrins**
S. Banfi¹, E. Caruso¹, E. Monti¹, M.B. Gariboldi¹, S. Alemani², G. Nasini² (¹Varese, ITA; ²Milano, ITA)
- PI81 Photostabilities of some photoactive organic species common in some sunscreens with different SPF**
 M. Tamam^{1,3}, A.M. Molokhia³, M.S.A. Abdel-Mottaleb^{1,2} (Cairo, EGY)

Wednesday, September 7, (14.00 – 15.30)
Poster session II
Raphaël

- PII1** **Enhanced selectivity of tri-component pro-drugs: enzyme specificity**
 E. Dickson, R. Goyan, J. Kennedy, K. Latulippe, R. Pottier, J. Wojtyk (Kingston, CAN)
- PII2** **Aggregation susceptibility on phototransformations of hematoporphyrin derivatives**
 P.F.C. Menezes, H. Imasato, V.S. Bagnato, J.R. Perussi (São Carlos, BRA)
- PII3** ***In situ* detection of singlet oxygen in intact HT29 cells by MALDI-TOF mass spectrometry**
M. Dodeller¹, B. Maunit¹, N. Lourette¹, L. Bezdetnaya², F. Guillemin², J.-F. Muller¹ (¹Metz, FRA; ²Nancy, FRA)
- PII4** **On the correlation between hydrophobicity, liposome binding and cellular uptake of porphyrin sensitizers**
S. Ben-Dror¹, I. Bronshtein¹, A. Wiehe², B. Röder², B. Ehrenberg¹ (¹Ramat Gan, ISR; ²Berlin, GER)
- PII5** **The thermodynamic effect of temperature and ionic strength on the binding of porphyrins to liposomes**
H. Weitman, R. Mines, I. Bloch, M. Gal, B. Ehrenberg (Ramat Gan, ISR)
- PII6** **Acid-base properties and liposome binding of a perfluoroalkylated phthalocyanine**
R. Mines¹, S.M. Gorun², B. Ehrenberg¹ (¹Ramat Gan, ISR, ²Newark NJ, USA)
- PII7** **Microscopic studies of cellular uptake and photocytotoxicity of hematoporphyrins in cancer cells. Effect of pH on the affinity, penetration depths and sensitization in membranes**
I. Bronshtein¹, T. Babushkina¹, Z. Malik¹, K.M. Smith², B. Ehrenberg¹ (¹Ramat Gan, ISR; ²Baton Rouge LA, USA)
- PII8** **Modularly synthesized porphyrazines with tuned hydrophobicity for near IR photosensitization**
A. Sholto¹, S. Lee², B.M. Hoffman², B. Ehrenberg¹ (¹Ramat Gan, ISR; ²Evanston IL, USA)
- PII9** **Interaction between porphyrins and filamentous phages as non-covalent supramolecular antenna system**
N. Angelini, M.A. Castriciano, A. Romeo, N. Micali, C. Lo Passo, I. Pernice, F. Felici, L. Monsù Scolaro (Messina, ITA)
- PII10** **Chiral recognition in bimolecular electron transfer between amino acids and photoactivated acceptors**
R.E. Galian, M.C. Morant-Miñana, L. Pastor-Pérez, J. Pérez-Prieto, M.Á. Miranda (Valencia, ESP)
- PII11** **Simultaneous determination of physical (k_q) and chemical (k_r) rate constants for singlet oxygen quenching using a steady-state IR luminescence technique**
C. Pierlot, J. Marko, J. Barbillat, J.-M. Aubry (Villeneuve d'Ascq, FRA)
- PII12** **Accumulation of sensitizer in rat embryos: spectroscopic studies**
 V. Legenis, A. Sukackaitė, V. Žalgevičienė, G. Gražlienė, J. Didžiapetrienė, R. Rotomskis (Vilnius, LTU)
- PII13** **Time-dependent self-assembly of 31-epimerically pure and mixed zinc methyl bacteriopheophorbides-*d***
T. Miyatake¹, K. Shitasue¹, Y. Omori¹, K. Nakagawa¹, M. Fujiwara¹, T. Matsushita¹, H. Tamiaki² (¹Otsu, JPN; ²Kusatsu, JPN)
- PII14** **A synthetic route to novel porphyrin - cyclam/cyclen conjugates for cancer therapy**
C. Welch, R.W. Boyle, S.J. Archibald (Hull, GBR)
- PII15** **Synthesis and *in vitro* investigation of cationic 5,15-diphenyl porphyrin-mono-clonal antibody conjugates as targeted photodynamic sensitizers**
 N. Pesa, K.A. Smith, H. Savoie, J. Greenman, R.W. Boyle (Hull, GBR)
- PII16** **Photodynamic inactivation of ion channels formed by mini-gramicidin in bilayer lipid membranes**
 Y.N. Antonenko¹, E.A. Dutseva¹, E.A. Kotova¹, J.R. Pfeifer², U. Koert² (¹Moscow, RUS; ²Marburg, GER)
- PII17** **Tetraazachlorins - new efficient near infrared photosensitizers for photodynamic therapy**
S.V. Barkanova, E.A. Lukyanets, E.A. Makarova, N.B. Morozova, L.V. Umnova, R. I. Yakubovskaya (Moscow, RUS)

- PII18 Improvement by solubilization in DMPC liposomes of PPME photodynamic effect – A study in human colon cancer cells HCT-116**
L. Delanaye¹, C. Volanti¹, N. Jacobs¹, R. Greimers¹, F. Tfibel², M.-P. Fontaine Aupart², A. Vanderplasschen¹, M. Hoebeke¹, J. Piette¹ (¹Liège, BEL; ²Orsay, FRA)
- PII19 Photodynamic therapy induces activation and translocation of HIF-1alpha as reported by green fluorescent protein *in vitro***
S. Mitra, S. Cassar, J. Puskas, J.G. Frelinger, T.H. Foster (Rochester NY, USA)
- PII20 Photosensitizer dosimetry reduce inter-subjects variation of photodynamic therapy treatment response**
X. Zhou¹, B.W. Pogue¹, B. Chen¹, E. Demidenko¹, P.J. Hoopes¹, T. Hasan² (¹Hanover NH, USA; ²Boston MA, USA)
- PII21 Aggressive tumours might be radiosensitized by porphyrins**
Ž. Lukšienė (Vilnius, LTU)
- PII22 PDT-induced changes in angularly resolved light scattering from intact cells as a reporter of mitochondrial and lysosomal morphology**
J.D. Wilson, T.H. Foster (Rochester NY, USA)
- PII23 Time dependent subcellular localisation of mTHPC and apoptotic response in photosensitized MCF-7 cells**
A. François, S. Marchal, F. Guillemin, L. Bolotine (Vandoeuvre-Lès-Nancy, FRA)
- PII24 Caspase-2: a possible trigger of apoptosis induced by ZnPc photodynamic treatment in A-549 cells**
 J. Cristobal, M. Cañete, A. Villanueva, S. Rello, A. Juarranz, J.C. Stockert (Madrid, ESP)
- PII25 Time-resolved singlet oxygen phosphorescence detection in cells using kHz diode-pumped solid-state lasers**
 S. Nonell (Barcelona, ESP)
- PII26 Binding of cationic porphyrin to double stranded viral DNA analyzed by comprehensive spectroscopic methods**
 K. Zupan¹, L. Herényi¹, K. Thót², Z. Majer¹, G. Csik¹ (¹Budapest, HUN; ²Heidelberg, GER)
- PII27 An ESR study on type I and type II photoreaction induced by neutral and cationic porphyrin derivatives**
M. Egyeki, G. Csik, P. Gróf (Budapest, HUN)
- PII28 Systemic suppression of contact hypersensitivity in mice induced by products of merocyanine 540 photolysis**
A.A. Kyagova, L.A. Kozir, E.A. Kozhinova, A.Ya. Potapenko (Moscow, RUS)
- PII29 Photothermal therapy of experimental tumours using Pd(II)- and Pt(II)-octabutoxy-naphthalocyanines as sensitizers**
M. Camerin¹, G. Jori¹, M.A.J. Rodgers², M.E. Kenney³ (¹Padova, ITA; ²Bowling Green OH, USA; ³Cleveland OH, USA)
- PII30 Antitumour properties of irradiated visible light active titanium dioxide photocatalysts**
A. Jańczyk, W. Macyk, K. Urbańska, G. Stochel (Kraków, POL)
- PII31 Nitric oxide-induced long-term protection of tumor cells against photooxidative killing**
M. Niziolek¹, W. Korytowski^{1,2}, A.W. Girotti² (¹Kraków, POL; ²Milwaukee WI, USA)
- PII32 Apoptosis accommodating effects of nitric oxide (NO) in photodynamically stressed tumor cells**
M. Niziolek¹, T. Krisak², W. Korytowski^{1,2}, A.W. Girotti² (¹Krakow, POL; ²Milwaukee WI, USA)
- PII33 Influence of aggregation, pH and environment on photostability of TPPS4: spectroscopic study**
J. Zerebcova, J. Valanciunaite, S. Bagdonas, G. Streckyte, R. Rotomskis (Vilnius, LTU)
- PII34 Application of N-acetyl-3,7-dihydroxyphenoxazine as singlet oxygen and hydrogen peroxide sensor in photodynamic reactions**
A. Ryabova, A. Stratonnikov, E.A. Lukyanets, V. Loschenov (Moscow, RUS)

- PII35 Heme oxygenase-1 protects tumor cells against PDT-induced toxicity**
T.A. Stoklosa¹, D. Nowis¹, M. Legat¹, T. Grzela¹, G. Wilczyński¹, E. Wilczek¹, A. Jalili¹, E. Głodkowska¹, P. Mrówka¹, M. Makowski¹, T. Issat¹, J. Dulak², A. Józkowicz², M. Adamek³, P. Nazarewski¹, M. Jakóbiśiak¹, J. Golab¹ (¹Warsaw, POL; ²Kraków, POL; ³Katowice, POL)
- PII36 On the role of calcium elevation in glioblastoma cell death under hypericin-induced photodynamic treatment**
D.E. Bragin¹, G. Pfaffel-Schubart², A. Rück² (¹Albuquerque NM, USA; ²Ulm, GER)
- PII37 Singlet oxygen generation by selected phthalocyanines and naphthalocyanines**
A.M. Edwards¹, M. Fajardo¹, M. Muñoz¹, G. Jori² (¹Santiago, CHI; ²Padova, ITA)
- PII38 Fluorescence spectroscopic study of hypericin-photosensitized oxidation of low-density lipoproteins**
S. Kascakova^{1,2}, M. Refregiers², D. Jancura¹, F. Sureau³, J.-C. Maurizot², P. Miskovsky^{1,4}
(¹Kosice, SVK; ²Orleans, FRA; ³Bobigny, FRA; ⁴Bratislava, SVK)
- PII39 Photothermal sensitisation of mammalian cells with nickel-octabutoxy-naphthalocyanine**
S. Rello-Varona, V. Moreno, Á. Villanueva (Madrid, ESP)
- PII40 Mechanisms of uptake of a water-soluble anionic zinc phthalocyanine in murine fibrosarcoma cells (RIF-1)**
M.M. Rashid, J. Griffiths, J. Schofield, S.B. Brown, D.I. Vernon (Leeds, GBR)
- PII41 m-THPBC potential as photosensitizer for liver PDT**
H.-P. Lassalle¹, F. Marchal¹, S. Marchal¹, M.A. D'Hallewin¹, F. Guillemin¹, J. Moan², L. Bezdetnaya¹
(¹Vandœuvre-les-Nancy, FRA; ²Oslo, NOR)
- PII42 Development of sensitizers based on squaraine moiety for photodynamic therapy**
D. Ramaiah¹, K.T. Arun¹, K. Jyothish¹, B. Epe² (¹Trivandrum, IND; ²Mainz, GER)
- PII43 QSAR modeling and prediction of tumoricidal activity of aryl-porphyrins in photodynamic therapy**
E. Papa, P. Gramatica, S. Banfi, E. Caruso (Varese, ITA)
- PII44 Halogenated water-soluble porphyrins and their photodynamic action in melanoma cells**
L.G. Arnaut¹, J.M. Dabrowski², C. Monteiro¹, A. Peixoto¹, M.M. Pereira¹, S.J. Formosinho¹, G. Stochel², K. Urbanska² (¹Coimbra, POR; ²Krakow, POL)
- PII45 Combined action of Visudyne and coherent or non-coherent light on melanoma cells**
P. Nowak-Sliwinska, G. Stochel, K. Urbanska (Kraków, POL)
- PII46 Complete model of oxygen transport in photodynamic therapy: a simulation of oxygen dynamics *in vivo***
K.K.-H. Wang, S. Mitra, T.H Foster (Rochester NY, USA)
- PII47 Preclinical evaluation of photodynamic therapy in new retinoblastoma xenografts**
I. Aerts¹, P. Leuraud¹, I. Laville¹, J. Blais¹, Ph. Maillard², L. Desjardins¹, M.F. Poupon¹, F. Doz¹ (¹Paris, FRA; ²Orsay, FRA)
- PII48 Synthesis and preclinical studies of targeted, two-photon activated photo-dynamic therapy agents**
J.R. Starkey, F. Meng, A. Gong, B.L. Moss, A. Rebane, M. Drobizhev, C.W. Spangler (Bozeman MT, USA)
- PII49 Photodynamic therapy for treatment of COPD. Clinical results**
N.E. Vasiliev (Novosibirsk, RUS)
- PII50 Peripheral benzodiazepine receptors and apoptotic response in REH-cells after ALA-PDT**
Ž. Lukšienė (Vilnius, LTU)
- PII51 Spectroscopic monitoring during ALA-PDT of human basal cell carcinoma**
W.J. Cottrell¹, T.H. Foster¹, A.R. Oseroff² (¹Rochester NY, USA; ²Buffalo NY, USA)
- PII52 ALA-PDT attenuates expression of chimeric oncoprotein BCR-ABL in chronic myelogenous leukemia cells K562 and disrupts the cytoskeleton structure**
M. Pluskalová, D. Grebeňová, K. Kuželová, P. Halada, Z. Hrkal (Prague, CZE)

- PII53 Photodynamic treatment with 5-aminolevulinic acid induces mitotic arrest in HeLa cells**
V. Moreno, A. Juarranz, J.C. Stockert, M. Cañete, S. Rello, A. Villanueva (Madrid, ESP)
- PII54 Ultraviolet-induced autofluorescence characterization of normal and tumoral esophageal epithelium cells**
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- PII84 A flavoprotein isolated from phototactic zoospores of a brown alga, *Scytosiphon lomentaria*: a new member of “Old Yellow Enzyme” family**
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D.C. Lührs¹, M.T.A. Alexandre¹, I.H.M. van Stokkum¹, R. Hiller², M. Groot¹, J.T.M. Kennis¹, R. van Grondelle¹ (¹Amsterdam, NED; ²Sidney, AUS)
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A. Espagne¹, P. Changenet-Barret¹, P. Plaza¹, K.J. Hellingwerf², M.M. Martin¹ (¹Paris, FRA; ²Amsterdam, NED)

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V.V. Rassadina¹, S.F. Koval², N.G. Averina¹ (¹Minsk, BLR; ²Novosibirsk, RUS)
- PII90** **Self-assembly of amphiphilic zinc chlorins possessing a hydrophilic oligooxyethylene group**
 T. Miyatake¹, T. Onishi¹, S. Kato¹, M. Fujiwara¹, T. Matsushita¹, H. Tamiaki² (¹Otsu, JPN; ²Kusatsu, JPN)
- PII91** **Supramolecular gels prepared with self-assembly of amphiphilic zinc chlorines**
 T. Miyatake¹, S. Tanigawa¹, E. Takenaka¹, M. Fujiwara¹, T. Matsushita¹, H. Tamiaki² (¹Otsu, JPN; ²Kusatsu, JPN)
- PII92** **Streptomycin effects on the activity of chlorophyll biosynthesis in barley seedlings**
E.B. Yaronskaya, E.R. Gritskevitch, N.G. Averina (Minsk, BLR)
- PII93** **Regulation of 5-aminolevulinic acid synthesis in roots of barley seedlings**
I.V. Vershilovskaya, E.B. Yaronskaya, N.G. Averina (Minsk, BLR)
- PII94** **Seasonal dynamics of xanthophyll cycle pigments in lichen *Xanthoria parietina***
H. Vráblíková¹, M. McEvoy², K.A. Solhaug², M. Barták¹, Y. Gauslaa² (¹Brno, CZE; ²Ås, NOR)
- PII95** **Photosynthetic characterization of the seagrass *Cymodocea nodosa* along depth and within leaf gradients**
I. Olivé, M.P. García-Sánchez, J.J. Vergara, J.L. Pérez-Lloréns (Cádiz, ESP)
- PII96** **Activation of photosynthetic electron transport and differential expression of proteins in rice (*Oriza sativa* L.) leaves by photocatalyst (TiO₂)**
S.C. Hong¹, A.C. Chang¹, P.G. Shin¹, S.H. Kim¹, K.S. Lee¹, C.W. Lee² (¹Suwon, KOR; ³Chongju, KOR)
- PII97** **Isolation and characterization of a novel photomorphogenic and circadian clock mutant in *Arabidopsis***
É. Kevei¹, P. Gyula¹, R. Tóth¹, B. Fehér¹, A. Viczián¹, L. Kozma-Bognár^{1,2}, A.J. Millar², F. Nagy¹ (¹Szeged, HUN; ²Edinburgh, GBR)
- PII98** **New light signalling component affecting circadian clock in *Arabidopsis thaliana***
B. Fehér¹, É. Kevei¹, P. Gyula¹, R. Tóth¹, V. Sokolova¹, L. Kozma-Bognár^{1,2}, A.J. Millar², F. Nagy¹ (¹Szeged, HUN; ²Edinburgh, GBR)
- PII99** **Analysis of the maize leaf proteome after various UV-B treatments of lines differing in UV-B sensitivity**
P. Casati^{1,2}, X. Zhang³, A.L. Burlingame³, V. Walbot¹ (¹Stanford CA, USA; ²Rosario, ARG; ³San Francisco CA, USA)
- PII100** **Expression of genes for early light inducible proteins under oxidative stress in barley**
E.N. Pogulskaya, N.P. Yurina (Moscow, RUS)
- PII101** **Photooxidative stress in barley leaves treated with Rosa Bengal**
 N.V. Shalygo, N.V. Kozel (Minsk, BLR)

PL1**NF- κ B, a key player in PDT-induced inflammatory response***J.-Y. Matroule, C. Volanti, J. Piette**CBIG, University of Liège, B-4000 Liège, Belgium*

Tumor eradication by photodynamic therapy (PDT) implies multiple cytotoxic processes relying on the nature of the photosensitizer used combined to several tumor features and experimental conditions. Although apoptotic or necrotic death is predominant in PDT anti-tumor properties, it is now well-established that the immune system also takes part in tumor elimination. In this context, several mediators (cytokines, chemokines,...) are secreted by the treated tumor and responsible for a local inflammation. NF- κ B is a key transcription factor governing the transcription of numerous genes involved in inflammation upon a large number of stimuli. It is therefore of major interest to understand how PDT modulates NF- κ B activity in order to potentiate its action. We sought to assess the NF- κ B response to a new photosensitizer: pyropheophorbide a methylester (PPME). Since tumor microvasculature plays an essential role in PDT, our approach consisted in photosensitizing colon cancer cells and endothelial cells. Unexpectedly, PPME photosensitization of colon cancer cells gave rise to a ROS-independent biphasic NF- κ B activation resulting from the stimulation of the IL-1 receptor and the production of ceramide by the acidic sphingomyelinase. In contrast, PPME-treated endothelial cells exhibited a single wave and slower ROS-dependent NF- κ B activation involving an unknown tyrosine kinase, demonstrating that PPME-mediated NF- κ B activation mobilized distinct signaling pathways relative to the cell type. Importantly, we also showed that NF- κ B has a central role in the release of mediators by the PDT-surviving cells. We recently showed that an active COX-2 is produced by PDT-treated cells, which could promote tumor recurrences. In conclusion, this lecture will exemplify the multiple implications of NF- κ B in the cellular response to PDT.

IL2**Base stacking and base pairing effects on electronic energy relaxation in DNA***C.E. Crespo-Hernández, B. Cohen, B. Kohler**Department of Chemistry, The Ohio State University, 100 W. 18th Avenue, Columbus, Ohio 43210, USA*

UV light is strongly absorbed by the bases in DNA, giving rise to excited states that can decay to deleterious photolesions. Although the structures of many of these photoproducts have been determined, little is known about how singlet excited states evolve to form photoproducts. In double-stranded DNA, bases are organized horizontally in base pairs and vertically in base stacks. These dual architectural motifs perturb the electronic structure of DNA in poorly understood ways through interbase electronic coupling. Using single- and double-stranded, defined-sequence oligonucleotides, we have investigated the effects of base stacking and base pairing on DNA photoprocesses by femtosecond pump-probe spectroscopy. Although considerable attention has been focused in the past on the possibility of excited-state proton transfer between complementary bases, our results show that vertical base stacking has the greatest influence on the fate of singlet excited states. In many base-stacked oligonucleotides, singlet excited states are observed with lifetimes that are orders of magnitude longer than those of monomeric bases. These long-lived states are assigned to excited dimer ("excimer") states that involve two bases stacked at the time of light absorption. By observing ground-state repopulation dynamics, we have measured the quantum yields of formation for these excimer states for the first time. In some sequence contexts, nearly every excitation decays to an excimer state. These findings have important implications for DNA repair. By effectively constraining electronic energy to one strand at a time, the probability of an interstrand crosslink is greatly reduced. The electronic structure of the B-form double helix may thus favor intrastrand lesions, which can be readily repaired by nucleotide excision repair using the undamaged strand as a template.

IL3**Formation and repair of bipyrimidine photoproducts in mammalian cells exposed to UVB and UVA radiations***T. Douki¹, S. Courdavault¹, E. Sage², C. Baudouin³, M. Charveron³, A. Favier¹, J. Cadet¹*¹*DRFMC/SCIB UMR-E 3 CEA-UJF, CEA-Grenoble, France;*²*CNRS UMR 20, Institut Curie, Orsay, France;* ³*Institut de Recherche Pierre Fabre, Laboratoire de Biologie Cellulaire, Toulouse, France*

Dimerization of adjacent pyrimidine bases within DNA is a major event in the etiology of solar carcinogenesis. The photoproducts generated upon absorption of UVB photons by thymine and cytosine bases include cyclobutane dimers (CPD) and (6-4) photoproducts (64PP) at each of the four possible bipyrimidine doublets. An assay based on the use of HPLC associated with tandem mass spectrometry was designed in order to individually quantify these lesions. Their distribution was found to be similar in isolated DNA and in a wide variety of cell types including Chinese hamster ovary cells and primary cultures of human skin fibroblasts and keratinocytes. TC and TT sites are more photoreactive than CT and CC doublets. In addition, the ratio between the yields of CPD and 64PP greatly depends on the pyrimidine bases involved. 64PPs were found to be efficiently repaired from human skin cells while high proportions of CPDs could persist in cells, even after recovery of the proliferation capacities of cells which were not eliminated from the cellular population by apoptosis. Interestingly, when cells were exposed to a combination of UVB and UVA radiations, but not to pure UVB, an efficient photoconversion of 64PPs into their Dewar valence isomers was observed. The latter lesions were found to be removed from the DNA of human skin cells at rates similar to those of 64PPs. UVA was also found to induce the formation of CPDs in larger yield than oxidative damage. Interestingly, neither 64PPs nor Dewar isomers were detected and the TT CPD was produced in a 10 times higher yields than corresponding cytosine-containing lesions. This distribution greatly differs from that observed in UVB-irradiated cells and thus rules out involvement of a direct excitation process. In addition, the CPD repair rate was lower in UVA- than in UVB-irradiated cells. Altogether, our observations strongly support that CPDs are the major and most persistent DNA lesions irrespectively of the type of UV radiation.

IL4**Potential role of oxidative DNA damage and repair in the development of malignant melanoma***S. Hoffmann-Dörr¹, W. Eiberger¹, R. Greiner², B. Volkmer², T.M. Rüdiger, J.P. Radicella⁴, B. Epe¹*¹*Institut of Pharmacy, University of Mainz, D-55099 Mainz, Germany;* ²*Dermatologisches Zentrum Buxtehude, D-21614 Buxtehude, Germany;* ³*Dept. of Dermatology, Boston University School of Medicine, Boston, USA;* ⁴*Département de Radiobiologie et Radiopathologie, CEA, 92265 Fontenay-aux-Roses, France*

Since the incidence of malignant melanoma is not correlated with the cumulative UV dose, but rather with the incidence of sunburns (i.e. inflammation), it has been suggested that oxidative DNA modifications (rather than UVB-induced pyrimidine dimers) could play a role in the etiology of this type of cancer. This assumption is also in accordance with our finding that the majority of oxidative DNA modifications induced by solar radiation are generated at wavelengths >400 nm via excitation of endogenous photosensitizers. To further analyse the relevance of oxidative DNA damage for melanoma induction, we have compared the generation of oxidative DNA base modifications in melanoma cells and normal skin fibroblasts obtained from the same patients. The results indicate that the malignant phenotype is generally associated with increased susceptibility to light-induced oxidative damage. Furthermore, melanin appears not to play a major role as either mediator or protective factor in the damage induction. The damage by visible light was associated with the generation of micronuclei, with higher efficiency in the melanoma cells than in the normal fibroblasts. Surprisingly, drugs such as chlorpromazine, although

photosensitizers themselves, inhibited the generation of oxidative DNA base modifications by solar radiation. The repair rates of oxidative DNA modifications induced by visible light were quite variable in the various melanoma cells that were analysed. In particular, one melanoma cell line was virtually completely repair-deficient, while the fibroblasts from the same patient repaired at a normal rate ($t_{1/2}$ approx. 4 h). Taken together, the data show that oxidative DNA damage induced by visible light has genotoxic consequences in melanoma cells and suggest that somatic mutations affecting the repair of oxidative DNA base modifications may contribute to increased vulnerability and thus accelerated malignant transformation.

IL5

Mechanisms of DNA damage induced inhibition of transcription

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The inhibition of transcription observed in cells after exposure to UV irradiation is believed to be the result of the stalling of the transcription machinery at sites of UV induced photolesions in transcriptional active genes. These DNA lesions are removed via a specialized repair pathway termed transcription-coupled repair (TCR). The biological importance of TCR to counteract transcription-blocking DNA damage is manifested in patients that suffer from the rare recessive progeroid disorder Cockayne syndrome (CS). The clinical phenotype of CS patients is severe and characterized among others by hypersensitivity to sunlight, mental retardation and premature aging. CS cells, deficient in TCR, are unable to recover RNA synthesis after UV irradiation and the absence of transcription resumption was therefore related to impaired removal of transcription blocking lesions. However, recent data led us to propose that the transcription defect in CS is also caused by a defect in the initiation of transcription.

We investigated the effect of UV-irradiation on transcription using an *in vitro* transcription system that allowed uncoupling of initiation from elongation events. Our results suggest that UV-induced transcription inhibition is at least partially due to repression of transcription initiation and not solely due to blocked elongation at sites of lesions.

Blockage of transcription elongation by UV induced photolesions was assessed by chromatin immunoprecipitation (ChIP) analysis using crosslinked normal and CS cells and antibodies against the elongating RNAPII (RNAPII α) and CS group B (CSB) proteins. These studies showed that RNAPII α and CSB were preferentially recovered in association with DNA fragments harbouring photolesions. Furthermore, our approach revealed a dependency on CSB in the assembly of a chromatin-bound TCR complex that includes repair factors, CSA-containing ubiquitin E3-ligase complex and chromatin remodellers/modifiers at lesion-stalled RNAPII α .

OC6

Photochemistry of 5-Halouracil containing DNA

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DNA is polymorphic and exists in a variety of distinct conformations. Duplex DNA can adopt a variety of sequence-dependent secondary structures, which range from the canonical right-handed B form through to the left-handed Z form. Triplex and tetraplex structures also exist. All of these unique conformations are assumed to play important biological roles in processes such as DNA replication, and gene expression and regulation. However, the biological roles associated with the different structural conformations of DNA are not well understood because of the short lifetime of appearance of each structure and the difficulty in

creating a system to demonstrate the DNA local structure. Developing a Z-stabilizing monomeric unit, the Z stabilizer, allowed us to understand the solution structure of Z-DNA and to reveal the specific 2' β -hydrogen abstraction that gives rise to the Z-form-specific 2' α -hydroxylation of the ^1U -containing Z-form under UV irradiation. We also investigated the photoreaction of 5-halouracil in the A-form, B-form, G-quartet, and protein-induced DNA kinks. Hydrogen abstraction by 2'-deoxyuridin-5-yl generated from 5-halouracil under irradiation was atom specific and highly dependent on the DNA structure. In addition, DNA-mediated charge transport chemistry was sensitive to the DNA structure and base pair π -stacking. We propose that the electronic properties of DNA are highly dependent on the orientation of π -stacking (i.e., A-, B-, and Z-form DNA have different electronic properties). Furthermore, experimental studies show that $^{\text{Br}}\text{U}$ -containing Z-DNA has a unique electronic property, and that charge-transfer from G to $^{\text{Br}}\text{U}$ occurs efficiently within the four-base π -stacks in Z-DNA.

OC7

UVB-induced CPD-retaining cells in SKH1-hairless mice are BrdU-retaining stem cells which are NER-proficient

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Exposure to UVB radiation causes DNA damage, which may lead to mutations and skin cancer. Cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts (6-4PP) are predominant types of UVB-induced DNA-damage. Within the murine skin, the nucleotide excision repair (NER) is effective in repair of 6-4PP, but not of CPD, which are mainly lost through epidermal cell turnover. Interestingly, during chronic low-level UVB exposure we found some basal cells in the epidermis that accumulated CPD. In this study we addressed the question of whether these CPD-retaining basal cells are defective in NER, which would imply retention of 6-4PP. We chronically exposed wildtype (SKH1), and NER-deficient (XPC -/-) mice to a low daily dose of UVB radiation (71 and 25 J/m² respectively). Using immunofluorescence, we studied the accumulation of CPD and 6-4PP. In the SKH1 mouse skin accumulation of CPD was found in the epidermal basal layer as well as in hair follicles. Accumulation of 6-4PP was not found in the SKH1 mouse skin. In contrast, in the XPC -/- mouse both CPD and 6-4PP accumulated. The CPD-retaining basal cells found in the SKH1 mouse skin co-localized with BrdU-label retaining cells, confirming a rarely dividing behaviour, i.e. stem cells. Application of TPA showed that CPD-retaining basal cells were still able to divide which means that these cells are viable and not in senescence. Taken together, our data indicate that CPD-retaining basal cells in SKH1 mouse skin are stem cells that are not defective in NER. Accumulation of CPD is solely due to the persistence of these rarely dividing cells. Evidently, this accumulation of DNA damage in stem cells is likely to play a crucial role in low-level UVB-induced skin carcinogenesis.

OC8

Daily UVB exposure stimulates the global nucleotide excision repair of cyclobutane pyrimidine dimers

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Cyclobutane pyrimidine dimers (CPD) constitute the main photoproducts formed by UVB. In response to these DNA damage, p53 blocks the cell cycle to allow cells to repair by nucleotide excision repair (NER). The NER is divided in two pathways: the global genome NER (GGNER) and the transcription-coupled NER

(TCNER). In normal human cells, CPD formed on the transcribed strand are removed 2 to 5 times faster than those formed on the non-transcribed strand. Is the repair rates of CPD faster for cells irradiated with just one acute dose of UVB, compared to cells irradiated daily using low doses, followed by an acute dose of UVB? For this study, we used normal skin fibroblasts and LF041 cell line, a p53 deficient cell line derived from Li-Fraumeni fibroblasts. We irradiated these cells with 35 J/m² UVB every 12 hours for 15 doses (chronic dose, a total of 525 J/m²) followed by one acute dose of 400 J/m² UVB. We compared with the same cell lines irradiated with only the acute dose of 400 J/m². Following the 400 J/m² UVB, we let the cells repair between 0 to 32 hours. The repair rates were then evaluated at each nucleotide position by ligation-mediated polymerase chain reaction (LMPCR) along the p53 gene. Concerning normal fibroblasts, the repair rates were 2 to 5 times faster on the non-transcribed strand for cells irradiated with the chronic doses and the acute dose, than for fibroblasts irradiated only with the acute dose. This repair acceleration rates after chronic doses is not present on the transcribed strand. Therefore, it appears that chronic UVB irradiation strongly induces GNER, whereas it has no effect on TCNER. Concerning the LF041 cell line, the same repair rates were observed following the chronic and the acute doses for both transcribed and non-transcribed strands. So, it appears that chronic doses of UVB induce GNER in a p53 dependent manner. Work supported by a grant from the National Cancer Institute of Canada, with funds from the Canadian Cancer Society.

IL9

ALA-PDT: the basics

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ALA-PDT is a modified form of photodynamic therapy (PDT), in that a precursor drug (5-aminolevulinic acid, ALA) is used to promote the body into synthesizing a natural porphyrin (protoporphyrin IX, PpIX) in the tumour area, which can then be used as a photosensitizer to eradicate surface lesions. The main advantage of this approach is that protoporphyrin IX has a natural clearance mode, and thus it is very difficult to overdose the patient with either too much light or too much drug. Its main limitation is related to the restricted depth of action, which is related to both the depth of ALA migration into the tumour tissue and by the amount of light that reaches into the tissue. The principal photochemical reaction involved in ALA-PDT is a photosensitization process in which the biosynthetically produced protoporphyrin IX is excited with blue or red light, leading to the formation of excited singlet oxygen via an energy transfer photosensitization mechanism. Subsequent to this photosensitization, the highly reactive oxidizing oxygen attacks double bonds in cell membranes that lead to an efficient process of cell killing. ALA-PDT is thus based on a deliberate perturbation (by the physician) of the biosynthetic pathway for heme. In general, ALA induces clinically significant PpIX in many of the tissues that line the body surfaces or body cavities. Tissues that show little or no PpIX production include striated smooth and cardiac muscle, dermis, blood vessels, nucleated blood cells and most other tissues of mesodermal origin. Malignant tissues often show much more production of ALA induced PpIX than do the corresponding normal tissue. Since PpIX is produced in the mitochondria, ALA-PDT often leads to cell death via an apoptotic process, with some contribution from necrosis.

IL10

The involvement of the proteasome in regulation of PpIX synthesis and ALA-PDT

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ALA-PDT efficacy is dependent on competent PpIX synthesis of the tumor cells, and this in turn is subject to the expression and activity of the third enzyme of the heme pathway, porphobilinogen deaminase (PBGD). As a general rule, high expression and activity of PBGD is characteristic of neoplastic cells, thus far regulated by the differentiation stage of the tumor. We have shown a dual sub-cellular localization of PBGD, in the cytosol and in the nucleus where it interacts with the nuclear Ran-BPM. Lead poisoning of ALA-dehydratase (ALAD), the second enzyme of the pathway, affects markedly PDT efficacy and unexpectedly reduces PBGD activity as well. Furthermore, Pb⁺⁺ is shown to activate markedly the proteasome activity *via* inhibition of ALAD. We demonstrate enhanced PBGD degradation resulting in reduced PpIX synthesis, due to the inhibition of Pb⁺⁺-ALAD and its regulatory role on the proteasomal complex. Heme, the end product of the pathway, is shown to specifically restrain proteasome activity resulting in elevated stability and activity of PBGD and accumulation of PpIX. In conclusion, the efficacy of ALA-PDT was correlated with proteasome inhibition/activation by Pb⁺⁺ or hemin, correspondingly, which resulted in decreased/increased activities of the porphyrin-synthesis enzymes. Thus, divergent inhibition or activation effects of the proteasome may affect the ALA-photodynamic therapy outcome.

IL11

Prodrug approaches in biomedical optics

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One of the most selective methods in photodynamic therapy (PDT) of neoplastic diseases known today involves the endogenous administration of 5-aminolevulinic acid (5-ALA) in order to stimulate the intracellular formation of the photosensitizer protoporphyrin IX (PpIX). Due to its outstanding selectivity for various human diseases, 5-ALA-mediated PDT has been assessed experimentally for the treatment of different medical indications. However, the limited bioavailability of hydrophilic 5-ALA has widely hampered the access of this methodology to daily clinical practice. Although researchers became aware of this drawback already early in the development of 5-ALA induced PpIX, it took several years to adapt well-established concepts in pharmaceutical science to this methodology since Kennedy et al. proposed the use of 5-ALA for therapeutic purposes in dermatology.

However, with the introduction of lipophilic derivatives of 5-ALA aiming at improving 5-ALA's local bioavailability, this research area has recently experienced a true revival. Today, one of these derivatives, 5-ALA methyl ester gained marketing authorization under the trade name Metvix® for the treatment of actinic keratosis and basal cell carcinoma in most Western countries. Another ester of 5-ALA, 5-ALA hexylester (HAL), has very recently obtained approval for the improved diagnosis of superficial bladder cancer in Europe and is currently under clinical assessment in a clinical phase III trial in the US.

The present presentation is aiming at reviewing the pharmaceutical concepts underlying the use of lipophilic 5-ALA derivatives in biomedical optics. Following a brief discussion of the chemical as well as biochemical bases, experimental data from pre-clinical as well as clinical studies will be presented. Furthermore, potentially interesting medical fields in which the use of properly formulated 5-ALA derivatives could be beneficial for the further progress of this methodology in biomedical optics will be pointed out. Finally, limiting factors for the use of simple 5-ALA esters will be discussed.

OC12**Photobleaching kinetics and epithelial distribution of hexaminolevulinat induced PpIX in the photodynamic treatment of rat bladder cancer *in vivo***

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Purpose: PDT of rat bladder cancer 2 and 3 H after the end of 1H intravesical instillation of 8 mM hALA induces tumor destruction with intact normal bladder epithelium and wall at 100mW/cm² and 20J/cm². While the normal bladder wall is destroyed at 16mM and 20J/cm², tumor necrosis is absent. To investigate this discrepancy we report the photobleaching kinetics and fluorescence distribution *in vivo*.

Materials and methods: all experiments were performed 2 and 3 h after the end of 1H intravesical instillation of hALA (8 & 16mM) on normal and tumor bearing rat bladders. PpIX was extracted chemically. Photobleaching kinetics were monitored by fluorescence spectroscopy before and during irradiation *in vivo*. Fluorescence confocal imaging was performed *ex vivo*.

Results: tumors at 8 and 16mM, exhibit comparable fluorescence intensities, 4 to 6 fold higher than normal bladders. Similar PpIX concentrations were obtained for both instillations. In normals PpIX fluorescence kinetics decrease exponentially with similar decay constants at 8mM (0,2750 J/cm²) and 16mM (0,2781 J/cm²), comparable to 0,2621 J/cm² for tumors at 8mM. Tumors at 16mM bleach two times faster (0,6268 J/cm²). Fluorescence imaging for normals at both concentrations and tumors at 8mM show bright spots, whereas tumors at 16mM exhibit a diffuse fluorescence pattern.

Conclusion: the discrepant PDT responses observed in tumors cannot be explained by different PpIX levels since comparable fluorescence and PpIX concentrations. The diffuse (16mM) vs localised (8mM) fluorescence distribution within tumors which coincides with faster photobleaching at 16mM could probably be responsible for the inversed photodynamic response.

OC13**Ultra-sensitive fluorescence imaging of 5-ALA induced Protoporphyrin IX in human glioblastoma cells**

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In comparison with cell lines from ovarian or breast cancer, U373-MG glioblastoma cells accumulated only moderate amounts of protoporphyrin IX (PP IX), but were most effectively inactivated by photodynamic treatment after incubation with 5-aminolevulinic acid (5-ALA). In addition, photodynamic efficacy was found to decrease with cell age and upon addition of the differentiating agents butyric acid or phenylbutyric acid as well as phorbol myristate acetate (PMA), a modulator of protein kinase C (PKC) activity. Only in the case of phenylbutyric acid or PMA this decrease correlated with lower intracellular PP IX amounts, whereas no change in PP IX formation was observed after addition of butyric acid. Therefore, it is suggested that intracellular location of PP IX may account for its high photodynamic efficacy in U373-MG glioblastoma cells as well as for a decrease of this efficacy with cell age or upon addition of butyric acid.

For measuring PP IX fluorescence either in whole cells or within their plasma membranes, conventional or total internal reflection fluorescence microscopy (TIRFM) was used. By application of low excitation light doses (below 0.2 J/cm) as well as a novel ultra-sensitive electron multiplying (EM)-CCD camera for detection, light induced reactions during PP IX imaging could be almost avoided. First results showed that PP IX fluorescence in U373-MG

glioblastoma cells mainly originated from cellular membranes, whereas in breast or ovarian cancer cells a rather granular fluorescence pattern was observed. Possible changes of this fluorescence pattern during cell aging or upon addition of differentiating agents are subject of present investigations. Measurements of different intracellular PP IX location are compared with different sensitivities towards photobleaching and different fluorescence decay kinetics. Cell differentiation might be an important factor that modulates efficacy of photodynamic treatment with 5-ALA.

OC14**5-ALA and 5-ALA derivative-mediated effects on gram negative bacteria**

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The spread of antibiotic multi-resistant bacterial strains is actually one of the most worrying threats to public health. One strategy to overcome this problem might be the use of photodynamic therapy (PDT). In the past, different photosensitizers (PS) have already been tested successfully on various pathogenic micro-organisms like bacteria (*E. coli*, *Staphylococcus*), viruses (Papilloma, Herpes), fungi or parasites (*Plasmodium*, *Leishmania*).

Since about one decade, the increasing emergence of 5-aminolevulinic acid (5-ALA) mediated PDT has been observed presumably due to numerous advantages over first generation PS. 5-ALA has no photoactive properties, but when given exogenously, acts as a precursor of photosensitive porphyrins predominantly in organisms that are characterized by a high metabolic turnover, such as cancer cells and bacteria. However, the 5-ALA's weak ability to cross biological barriers has lead to the increased utilisation of more lipophilic derivatives like 5-ALA methyl or hexyl ester, who present an improved capacity to reach the cytoplasm.

Different studies have shown that Gram positive bacteria are significantly more sensitive to PDT than Gram negative strains. This has been attributed to a less sophisticated barrier of Gram (+) when compared to the complex multilayer barrier of Gram (-). Starting from the hypothesis that more lipophilic compounds with a positive charge under physiological conditions will cross the bacterial multilayer barrier with more ease, we have tested the efficacy of 5-ALA derivatives for bacterial inactivation. 5-ALA methyl ester was the most effective compound with respect to photodynamic inactivation of bacteria. Furthermore, the experiments showed significant differences in term of drug optimal concentration, bactericidal activities and porphyrin type production.

OC15**Photosensitization response measured by reflectance spectroscopy**

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Endogenous porphyrins were induced in mouse skin *in vivo* by topical application of 5-aminolevulinic acid (ALA) or deferoxamine (DF). After application of DF the recorded fluorescence spectra were similar to that of protoporphyrin IX (PpIX) induced by ALA. Reflectance spectroscopy of the skin seems to be a useful method to monitor changes in cutaneous oxyhaemoglobin and deoxyhaemoglobin. Exposure of mouse skin to ALA (0.1 mmol/g, 24 h) and light (632 nm, diode laser, 20 J/cm² at 100 mW/cm²) resulted in disappearance of oxyhaemoglobin peaks in the treated area, showing that oxygen was consumed and that blood vessel occlusion has taken place. After exposure of the skin to DF (0.3 mmol/g, 24 h), which induced about 5% of the ALA effect with respect to PpIX formation, and light (632 nm, diode laser, 50 J/cm² at 100 mW/cm²) practically no changes of the oxyhaemoglobin status were observed. In

conclusion, the present study shows that reflectance spectroscopy is a powerful method to monitor photodynamic effects on skin.

OC16

Phototoxic activity of 5-aminolevulinic acid-induced protoporphyrin IX against *Leishmania major*

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Leishmaniasis, a parasitic disease, is endemic in 88 countries with an estimated yearly incidence of 1-1.5 million cases of cutaneous leishmaniasis (CL). Despite the various existing anti-parasitic medications, the number of cases has not decreased. Up until now, there have been a few clinical reports showing that photodynamic therapy (PDT) with 5-aminolevulinic acid-induced protoporphyrin IX (ALA-PpIX), also known as ALA-PDT, was effective as a treatment of CL. However, no detailed observation has been done *in vitro*. In this study, we investigated the cellular mechanism of ALA-PDT for CL. After co-incubation of *L. major* promastigotes and 0.1 μM of ALA, the ALA-PpIX level was extremely low (7.03×10^{-16} mol/parasites) compared to the PpIX level after co-incubation with 0.1 μM PpIX (2.72×10^{-15} mol/parasites, $p < 0.001$). The maximum ALA-PpIX level was seen at 6 h of incubation, and then the level gradually decreased. In comparison, the ALA-PpIX level showed a dose-dependent pattern in J774 cells and bone marrow-derived macrophages. There was no difference in the ALA-PpIX level between uninfected and infected cells (co-incubation with *L. major* for 24 h at 1:10), although infected cells were more resistant than uninfected cells. The cell survival rate 24 h after irradiation with 635 nm laser at $10\text{J}/\text{cm}^2$ was $91.9 \pm 16\%$ for infected cells and $32 \pm 28\%$ for uninfected cells. Comparative analysis of intracellular distribution of PpIX by confocal laser scanning microscopy revealed no difference between infected and uninfected cells; ALA-PpIX mainly localized in the cell membrane and mitochondria. Exposure of the J774 cells to different agents, such as all-trans retinoic acid (0.1-1 μM), 13-cis retinoic acid (1-10 μM), and 1,25-(OH) $_2$ -D $_3$ (2-20nM), did not increase intracellular ALA-PpIX levels. *Leishmania* cannot produce ALA-PpIX due to a deficiency of several enzymes in the heme synthetic pathway. It is likely that macrophages, which phagocytize *Leishmania*, produce ALA-PpIX and are damaged by PDT. Thus, a good clinical outcome of ALA-PDT is probably not due to the direct parasite killing effect.

IL17

The genetics of ROS-mediated stress responses in *Arabidopsis*

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The evolution of aerobic metabolic processes such as respiration and photosynthesis unavoidably lead to the production of reactive oxygen species (ROS) in mitochondria, chloroplasts and peroxisomes. A common feature among the different ROS types is their capacity to cause oxidative damage by inactivations e.g. proteins, nucleic acids and lipids. These cytotoxic properties explain the evolution of complex arrays of ROS scavengers. In plants chloroplasts and peroxisomes are the major sites of ROS production. Various abiotic stress conditions may limit the ability of a plant to use light energy for photosynthesis. Under such stress conditions hyper-reduction of the photosynthetic electron transport chain and photoinhibition of photosynthesis may occur even at moderate light intensities, often causing damages that have been interpreted as unavoidable consequences of injuries inflicted upon plants by toxic levels of ROS. However, this paradigm needs to be modified. Stress responses triggered by ROS are not only due to physicochemical damages but may also be caused by the activation of genetically determined stress response programs. We'll present and comment data from the work of several groups suggesting that ROS may act as signals whose specificities seem to depend on the

chemical identity of a given ROS and its intracellular site of generation.

IL18

Crosstalk and antagonistic response to singlet oxygen and other reactive oxygen species in *Arabidopsis thaliana*

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During abiotic stress conditions like high light exposure, different reactive oxygen species (ROS) are generated simultaneously in plants, making it difficult to determine the biological activity and mode of action for each of these ROS separately. Such a study requires finding conditions under which only one specific ROS is generated. In order to address this problem, we made use of the conditional *flu* mutant of *Arabidopsis thaliana* that accumulates the photosensitizer protochlorophyllide (Pchl id) in the dark, to generate singlet oxygen ($^1\text{O}_2$), a non-radical reactive oxygen species, in plastids in a controlled and noninvasive manner. Within the first minute of re-illumination, the excited triplet $^3\text{Pchl}id$ interacts with O_2 to generate $^1\text{O}_2$. Rapidly after the release of $^1\text{O}_2$, *flu* plants stop growing and initiate a cell death response. By using Affymetrix Genechip microarrays we have shown that $^1\text{O}_2$ is involved in activating distinct sets of early stress-response genes that are different from those activated by superoxide (O_2^-) / hydrogen peroxide (H_2O_2) during a treatment with paraquat, an herbicide that acts as a terminal oxidant of photosystem I. The up-regulation of genes selectively activated by $\text{O}_2^-/\text{H}_2\text{O}_2$ -but not by $^1\text{O}_2$ - was strongly suppressed in plants over-expressing thylakoidal ascorbate peroxidase (tAPX). Surprisingly, the over-expression of tAPX in the *flu* mutant increased the extent of $^1\text{O}_2$ -induced cell death and the up-regulation of genes selectively activated by $^1\text{O}_2$. These results suggest that $\text{O}_2^-/\text{H}_2\text{O}_2$ antagonize the $^1\text{O}_2$ -mediated stress response and highlight the necessity of considering different ROS separately rather than as a whole.

IL19

Genome-wide analysis of photorespiratory hydrogen peroxide-regulated gene expression in *Arabidopsis*

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In plants, reactive oxygen species and, more particularly, hydrogen peroxide (H_2O_2) play a dual role as both toxic by-products of normal cell metabolism and regulatory molecules in stress perception and signal transduction. Peroxisomal catalases are an important sink for H_2O_2 . By using ATH1 Affymetrix microarrays, expression profiles were compared between control and catalase-deficient *Arabidopsis thaliana* plants. Reduced catalase levels already provoked differences in nuclear gene expression under ambient growth conditions and these effects are amplified by high light exposure in a sun simulator for 3 and 8 h. Genome-wide expression analysis allowed the characterization of complete pathways and functional categories during H_2O_2 stress. In total, 349 genes were significantly upregulated by photorespiratory H_2O_2 and 88 were downregulated. From this data set, H_2O_2 was inferred to play a key role in the transcriptional upregulation of small heat shock proteins during high light stress. In addition, several transcription factors and candidate regulatory genes involved in H_2O_2 transcriptional gene networks were identified. Comparisons with other publicly available transcriptome data sets of abiotically stressed *Arabidopsis* revealed an important intersection with H_2O_2 -deregulated genes, positioning elevated photorespiratory H_2O_2 levels as an important signal within abiotic stress-induced gene expression. Finally, by analyzing transcriptional changes in a combination of a genetic (catalase deficiency) and an

environmental perturbation (high light) a transcriptional regulon was identified that was strongly and rapidly induced by high light in control plants, but impaired in catalase-deficient plants. The regulon encloses the complete known anthocyanin regulatory and biosynthetic pathway, together with genes of hitherto unknown function.

IL20

Molecular biology of programmed cell death in *Arabidopsis thaliana*

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Hydrogen peroxide (H₂O₂), generated by various environmental and developmental stimuli, can act as a signaling molecule that regulates plant development, stress adaptation and programmed cell death (PCD). H₂O₂-induced PCD itself is essential for a number of developmental processes and environmental responses, including aleurone cell death, the hypersensitive response to pathogens, and allelopathic plant-plant interactions. The mechanisms of H₂O₂ generation and detoxification are well-studied but little is known as to how the H₂O₂ signal is perceived and then channeled downstream the signaling network in order to achieve the regulation of these processes. To address this question, a novel system for studying H₂O₂-induced programmed cell death in *Arabidopsis thaliana* was developed. The catalase inhibitor aminotriazole (AT) reduced the catalase activity and caused endogenous accumulation of hydrogen peroxide that eventually triggered PCD. Microarray analysis with a DNA chip representing 21500 genes and subsequent comparison with other PCD-related expression studies revealed a set of new H₂O₂-responsive genes that were highly regulated in a common fashion during different types of PCD. These included an oxoglutarate-dependent dioxygenase and various oxidoreductases, the transcription factors Zat11, WRKY75 and NAM, proteasomal components, a heterologous group of genes with diverse functions, and genes encoding proteins with unknown functions. A number of them are being functionally studied by knockout technology and by generating plants with altered gene expression. In addition, our group has isolated mutants that are more tolerant to AT as well as to the fungal AAL toxin, thus indicating a new link between redox and sphingolipid signaling. These genetic studies were further substantiated by molecular and biochemical data bringing new insights into the interplay between H₂O₂ and sphingolipids during PCD.

OC21

Ultraviolet illumination induces a stress effect upon higher plants (*Spirodela oligorrhiza*), as evident by the universal stress signal, alanine – an ¹⁵N NMR study

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¹⁵N Nuclear Magnetic Resonance spectroscopy was used to follow nitrogen metabolism and amino acid production in etiolated *Spirodela oligorrhiza*. Plants were exposed to either complete darkness or to 15 min pulsed U.V. illumination and fed with 30mM ¹⁵N-enriched ammonium chloride (added to Hutner's growth medium containing 0.5% sucrose) for 24h. Their ¹⁵N enriched amino acid pattern was compared.

The ¹⁵N NMR analysis revealed ¹⁵N incorporated mainly into glutamine and asparagine. However, when plants were exposed to U.V. illumination, alanine was also produced.

A literature survey reveals a number of previous reports of alanine production (among other metabolites) in response to **stress**. The

general conclusion is drawn, based on present and previous results (Monselise *et al* 2003), that *alanine accumulation is a universal first stress signal in a wide variety of organisms*, an observation not previously realized. Evidence of formation of "heat shock proteins" and enhanced protein kinase activity under various stress conditions has been reported. We propose that enhanced alanine production and accumulation precedes this stress activity as a general first stress signal.

Reference: Monselise, E. B-I, Parola, A. H. and Kost, D. (2003) Low Frequency Electromagnetic Fields Induce a Stress Effect upon Higher Plants, as Evident by the Universal Stress Signal, Alanine. Biochemical Biophysical Research Communication (BBRC) 302 427 – 434.

OC22

Phototoxic phytoalexins: a new mechanism of light-mediated plant defence

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Plants protect themselves through a wide array of constitutive and induced defence mechanisms, which include programmed cell death, surface-to-air signalling, expression of defence proteins, and production of antimicrobial secondary metabolites. The latter compounds can be divided into two groups: phytoalexins, which are synthesized *de novo* upon biotic or abiotic stress, and phytoanticipins, which are pre-formed antimicrobial metabolites.

Phytoalexins have several interesting characteristics: (1) Prior to infection, they are generally undetectable in the plants. (2) They are synthesized very rapidly, within hours following microbial attack or stress situation. (3) Their formation is restricted to a local region around the infection site. (4) They are toxic to a broad spectrum of fungal and bacterial pathogens of plants. Globally, phytoalexin production represents a very economical way to counteract hazard, because the carbon and energy resources are diverted to phytoalexin synthesis only at the early period of attack, and only at its site. Moreover, this strategy avoids autotoxicity until a challenge arises, since the biological activity of these compounds is inherent in the definition of phytoalexins.

Herewith we report on the photophysical and photobiological studies on phenalenone phytoalexins. We show that these secondary metabolites are able to photosensitize the production of singlet oxygen with concomitant toxicity against their eliciting pathogen, e.g. the fungus *fusarium oxysporum*, upon exposure to light and oxygen. The role of the phenalenone chromophore and, more fundamentally, of singlet oxygen in plant defence is discussed.

Acknowledgements: this work has been supported by the Spanish Ministerio de Ciencia y Tecnología (SAF 2002-04034-C02-02).

IL23

TbWC-1: the photoreceptor of an hypogeous fungus

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Ectomycorrhizal fungi, such as the filamentous ascomycete *Tuber borchii*, are plant-symbiotic microorganisms that colonize most trees and shrubs in temperate forests, thereby improving their growth performance as well as resistance to a variety of abiotic and biotic stresses.

Central to the colonization process is the formation of a highly specialized structure, the ectomycorrhiza, where the exchange of nutrients (and other signals) between the fungus and the root system takes place.

Here we present evidence for the influence of light on *Tuber borchii* mycelial growth and the identification and cloning of a gene, *Tbwc-1*, homologous to a blue-light photoreceptor of *Neurospora crassa*. As in the case of *N.crassa*, blue-light

irradiation of *T.borchii* colonies inhibits their apical growth. In *Neurospora*, the response is controlled by a nuclear photoreceptor, NcWC-1 (White Collar-1), which consists of a sensor domain (LOV) and a transcriptional factor moiety.

We isolated a gene (*Tbwc-1*) whose deduced amino acid sequence shows a high similarity and colinearity of domains with NcWC-1, except for the polyglutamine regions. As previously found in *Neurospora*, *Tbwc-1* mRNA is under light control and its steady level increases upon irradiation.

TbWC-1 LOV sensor domain has all the residues necessary for flavin binding, among which Cys334 is essential for the formation of a cysteinyl adduct and for the entry into a photocycle, supporting the hypothesis that TbWC-1 is a photoreceptor.

IL24

Multiple signal chains in the blue light dependent regulation of photosynthesis genes in *Rhodobacter sphaeroides*

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We have investigated how blue light affects gene expression in the facultatively photosynthetic bacterium *Rhodobacter sphaeroides*. *R. sphaeroides* forms photosynthetic complexes only when oxygen drops below a certain threshold value. At intermediate oxygen concentration blue light inhibits the formation of photosynthetic complexes. We have shown that the BLUF domain (Gomelsky and Klug, 2002) protein AppA is a photoreceptor for blue light (Braatsch et al., 2002). AppA functions as an antagonist of the PpsR protein that represses expression of photosynthesis genes under high oxygen tension. AppA integrates light and redox signals. As a consequence, expression of photosynthesis genes is repressed when oxygen and blue light are present. Our work also demonstrated that the BLUF domain can function as a module and relay signals to different output domains (Han et al., 2004). In the absence of oxygen, however, blue light stimulates the expression of photosynthesis genes. This signal is mediated by the photosynthetic electron transport. An electron transport dependent signal is recognized by the PrrB/PrrA two component system that activates photosynthesis genes under low oxygen tension and dominates the repressing effect by the AppA/PpsR system. A transcriptome analysis indicated that additional blue light photoreceptors are involved in the regulation of gene expression in *R. sphaeroides* (Braatsch et al., 2004). The identity of these additional photoreceptors will be discussed. Thus, several light dependent signal chains exist in *Rhodobacter sphaeroides* that allow the appropriate adaptation to changing oxygen and light conditions.

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IL25

Signal transduction mechanism of the cryptochrome blue-light photoreceptor

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Cryptochromes are flavoprotein blue-light photoreceptors that modulate growth and adaptive responses in organisms ranging from bacteria to animals. Although they exhibit significant structural homology with the light-dependent DNA repair enzyme photolyase, whose photocycle is well characterized, their mechanism of action is unknown. Genetic and biochemical data from both plants and animals suggest that C-terminal domains

ranging in length from 30-250 amino acids beyond the photolyase-homology domain are involved in light-dependent signal transduction by cryptochromes. Using computational, biophysical and biochemical approaches, we demonstrate that isolated C-terminal domains from plants and animals are intrinsically unstructured peptides. However, stable intraprotein interactions between the photolyase-homology and C-terminal domains induce structure within the disordered domain under dark conditions. Irradiation of cryptochrome with light results in a conformational change that involves release of the disordered C-terminal domain from the globular protein. Possible consequences of this structural plasticity regarding the mechanism of action of cryptochromes will be discussed.

IL26

Effect of mutations on interdomain communication in the phototropin related *Bacillus subtilis* protein YtvA

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Mutagenesis studies on the phototropin- (phot) related protein YtvA from *Bacillus subtilis* have revealed the role of selected structural elements in interdomain communication. The LOV (Light, Oxygen, Voltage) domain of YtvA undergoes light-driven reactions similar to that of phot-LOV, with reversible formation of a flavin-cysteine covalent adduct. The mutated proteins YtvA-E56Q and YtvA-E105L have been studied by the fluorescence of the conserved W103 and by circular dichroism (CD) spectroscopy in the UV region between 190 and 250 nm. The E56Q mutation breaks the surface-exposed E56-K97 salt bridge, a structural element highly conserved within the LOV series. The E105L mutation is located at the solvent exposed surface of the central β -sheet, which has recently been shown to interact with an helical extension C-terminal to a phot-LOV2 core. CD data show that the mutation E105L affects the protein secondary structure, with the α -helix content decreasing to ca. 22% (compared to 33% in YtvA-WT) in the dark, and upon formation of the photoadduct results in larger conformational changes than in YtvA-WT. W103 becomes slightly more shielded from the solvent. On the contrary in YtvA-E56Q the CD spectrum is the same as in YtvA-WT, whereas W103 becomes more exposed to the solvent and the dark-recovery kinetics is slower. These results indicate that the E56-K97 salt bridge stabilizes locally the protein structure and participates in the regulation of the photocycle, but has negligible effects on the overall structure. The E105L mutation, instead, highlights the involvement of the central β -sheet in the light-driven conformational changes in LOV proteins.

OC27

Functional expression of algal light-sensitive adenylyl cyclases in animal cells

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The photoactivated adenylyl cyclase (PAC) is a recently purified and cloned flavoprotein complex (M. Iseki, S. Matsunaga, A. Murakami, K. Ohno, K. Shiga, C. Yoshida, M. Sugai, T. Takahashi, T. Hori, M. Watanabe [2002] Nature 415: 1047-1051). It represents a novel blue-light receptor mediating photomovements of the unicellular photosynthetic flagellate *Euglena gracilis*. The purified protein complex, apparently a heterotetramer consisting of two subunits (PAC α PAC β), shows adenylyl cyclase activity which is strongly enhanced upon irradiation with blue light. Iseki *et al.* (2002) already pointed out its biotechnological utility to control cAMP-regulated processes by light irradiation. We have now functionally expressed PAC α and PAC β in oocytes from *Xenopus*

laevis and show that both proteins are independent blue light-activated enzymes. Cytoplasmic cAMP increase was measured electrically via co-expressing the human epithelial chloride channel CFTR (cystic fibrosis transmembrane conductance regulator) which is activated by endogenous cAMP-dependent protein kinase. The action spectra of both, PAC α and PAC β , are FAD-like, confirming that both proteins are FAD-based light sensors. Inactivation of the N-terminal FAD-binding domain (F1) rendered PAC α inactive, whereas inactivation of the F2-domain had no obvious influence on the activity of PAC α . The expression of PAC α and PAC β was independently demonstrated with western blots. The intracellular cAMP concentration in single PAC-expressing oocytes was additionally detected with a cAMP enzyme immunoassay.

OC28

Light-induced multi-step intra-protein electron transfer in DNA photolyase and cryptochrome

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Photolyases and cryptochromes form a class of structurally tightly related flavoproteins that serve quite different functions. Photolyases repair UV-induced DNA lesions using the energy of near UV/blue photons in a variety of organisms including, e.g., bacteria, plants or some mammals. Cryptochrome blue light receptors, on the contrary, cannot repair DNA but are implicated in growth regulation in plants and in the circadian clock of both plants and animals.

The two proteins share both a common cofactor, FAD (flavin adenin dinucleotide), that is found in different redox states in the two enzymes (oxidized in cryptochromes and semireduced in isolated photolyase) and a highly conserved chain of three tryptophans linking this cofactor to the enzyme's surface.

We used transient absorption spectroscopy to study wild type DNA photolyase from *E. coli* and cryptochrome1 from *A. thaliana* as well as some mutants with tryptophans of the chain replaced by redox inert phenylalanin and established the functioning of this special tryptophan chain for light-induced multi-step intra-protein electron transfer toward the FAD in both photolyase and cryptochrome. This is remarkable as the FAD cofactor is activated by light of different wavelength in the two cases. It is therefore intriguing to speculate that the electron transfer activity present in photolyases contains latent signaling potential that has been co-opted for a role in blue-light photoreceptors in the course of evolution.

IL29

Molecular targets of photodynamic therapy and cell death pathways: Traditional vs. novel linkages between them.

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Most photosensitizers for PDT are hydrophobic and membrane localizing. The critical molecular targets of PDT must reside very near to the cellular membrane sites of photosensitizer binding, since photodynamically-generated singlet oxygen reacts very near to the site of its formation. When PDT is sensitized by the phthalocyanine Pc 4 or certain other photosensitizers that localize in mitochondria and endoplasmic reticulum, important targets are the anti-apoptotic proteins Bcl-2 and Bcl-xL, which suffer a type of photodamage that is readily detected on western blots. We have been characterizing the initial damage to those proteins and associated structures and the subsequent cell death pathways that are triggered. We find that the primary lesions are more important in the killing of PDT-treated cells than are the late stages of apoptosis. Bcl-2 and Bcl-xL can interact with the permeability transition pore complex, which forms at contact sites between the inner and outer mitochondrial

membranes and provides a mechanism for release of cytochrome c from the mitochondria. These complexes and the inner membrane itself contain the unique phospholipid cardiolipin (CL). Our observation of fluorescence resonance energy transfer (FRET) from the CL probe nonyl-acridine orange (NAO) to Pc 4 indicates that Pc 4 binds within a few nm of CL and may also target that phospholipid. Although the mitochondrion appears to be central to cell killing by PDT, simultaneous damage to other organelles may amplify the lethal response. PDT also produces photodamage to ER proteins controlling the subcellular distribution of calcium ion, and PDT damage to lysosomes releases components, such as cathepsins, that can variously promote the mitochondrial pathways of cell death. Thus, damage to multiple cell organelles may signal to the mitochondrion to amplify apoptosis or necrosis initiated by mitochondrial damage.

IL30

Therapeutic exploitation of the tumor-protective mechanisms induced by the photodynamic therapy

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Photodynamic therapy (PDT) is an approved treatment modality used in the management of solid tumors. It is a two-phase treatment consisting of administration of a photosensitizer followed by tumor illumination with a visible light. Depending on the photosensitizer, fluence rate or endogenous physico-chemical characteristics of the treated tissue, tumor cells undergo apoptosis or necrosis. However, increasing evidence indicates that suboptimally treated regions of the tumor can respond by rescue responses thereby leading to insufficient cell death. The surviving cells might be the cause of relapse rendering the treatment less effective. Therefore, elucidation of molecular changes in the treated cells as well as identification of drugs that might interfere with rescue responses becomes an important area of investigation. PDT leads to the expression of heat shock proteins and numerous enzymes such as superoxide dismutase 2 (SOD-2), cyclooxygenase 2 (COX-2), heme oxygenase 1 (HO-1) that participate in the management of oxidative stress-induced damage. Some of these repair mechanisms can be targeted by specific drugs that could be used in combination treatment. Additionally, the unique mechanism of tumor destruction resulting from apoptotic and necrotic killing of tumor cells accompanied by local inflammatory reaction and induction of heat shock proteins might be exploited for the generation of effective immunotherapeutic strategies. Such combined treatment might induce systemic antitumor immunity and obviate one of the inherent limitations of PDT i.e. treatment of localized disease.

IL31

Role of endoplasmic reticulum-Ca²⁺ emptying and multidomain pro-apoptotic BAX and BAK proteins in shaping cell death after photodynamic therapy

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Photodynamic Therapy (PDT) is an anticancer therapy that uses a photosensitizing agent activated by visible light to generate reactive oxygen species (ROS), which destroy the cancer cells. Both the commitment event and the modality of cell death in photodynamic therapy (PDT) remain undefined. We report that PDT with the endoplasmic reticulum (ER)-associating photosensitizer hypericin leads to an immediate loss of sarco(endo)plasmic-reticulum Ca²⁺-ATPase (SERCA) 2 protein levels, causing disruption of Ca²⁺ homeostasis and cell death. Protection of SERCA2 protein by increasing the antioxidant capacity of the cells by chemical or genetic means rescues ER-Ca²⁺ levels and prevents cell death, suggesting that SERCA2 photodestruction with consequent incapability of the ER to maintain intracellular Ca²⁺ homeostasis causes cell killing. Apoptosis is rapidly initiated after ER-Ca²⁺

depletion and strictly requires the BAX/BAK gateway at the mitochondria. *Bax^{-/-}Bak^{-/-}* double-knockout (DKO) cells are protected from apoptosis but undergo a caspase-independent autophagic-cell death as revealed by electron microscopy and biochemical analysis. Re-expressing mitochondria-targeted BAX in DKO cells reconstitutes caspase-dependent apoptosis after PDT as observed in wild type cells. Autophagy inhibitors protect DKO cells from photokilling suggesting that autophagy is a death pathway. Thus following disruption of Ca²⁺ homeostasis, the presence or absence of BAX and BAK critically determines the modality of PDT-mediated cell killing: either apoptosis or autophagy.

IL32

Dissecting cellular and molecular events in PDT in combination with chemotherapy: additivity and synergy

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We compared the effects of mono-therapy (PDT or Chemotherapy) vs combination therapy (PDT plus Chemotherapy) on two human cell lines, namely the p53-null “non small cell lung cancer” H1299 and the p53-positive mammary MCF-7 cells.

The study was aimed at evaluating if this combination causes effects such that it can be hypothesized a reduction of cytostatic dosage without loss of overall efficacy, and in the case of a positive answer, at getting insight into molecular events involved. PDT was performed by irradiating Photofrin-preloaded H1299 cells with a suitable lamp or Indocyanine green-loaded MCF-7 cells with an infrared laser source. The cytotoxic drugs were CDDP (with H1299 and MCF-7) and Gemcitabine (with H1299 only).

To this purpose we have evaluated initially the consequences produced by PDT or chemotherapy administered singly and then those produced by combination therapy. In any condition, the effects were evaluated by comparing the cell viability (Trypan blue or MTT assays), cell cycle patterns and expression profiles of some key proteins (WB). In the case of MCF-7, we have also comparatively evaluated some metabolic cellular activity including cell growth (Thymidine incorporation), protein synthesis (Methionine incorporation) and glucose transport (insulin end non-insulin dependent).

Statistical treatment of both viability and metabolic data (Median (H1299) and Isobolographic (MCF-7) analyses), indicated mutual strengthening of therapeutic efficacy that could be converted to synergy using specific blends of PDT and Chemotherapy. However, the conditions that bring to synergy in responsive cells are related to drug ability to synchronize cells in a specific phase in which they are more sensitive to PDT. Concentrations of CDDP as low as 2.5 μM, kill preferentially cells crossing the S phase and induce accumulation of surviving cells in the G₀/G₁ phase. Cells resting in this phase are easily abrogated by PDT (either Photofrin or Hypericin) that kills preferentially cells in this phase. At variance, low Gemcitabine concentrations kill cells crossing the G₀/G₁ phase (as PDT): case the best attainable result is additivity. So, if an appropriate drug is used, and the PDT appropriately tuned, the cytostatic dose can be reduced without compromising the therapeutic response.

OC33

Decrease in adhesion of prostate cancer cells following subcurative photodynamic therapy

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PDT appears to hold promise for the treatment of localized recurrent prostate cancer. However, long-term effects of this treatment remain to be evaluated. The **objective** of this study is to evaluate the cellular/molecular response of prostate cancer cells

that have been exposed to both PS and light but not enough to kill them.

We used MatLyLu cells a highly metastatic rat prostate cancer line. [140 nM] BPD was used *in vitro*, 0.25 mg/kg of Verteporfin was used *in vivo*. Treatments were performed 1h after PS delivery with a 690 nm laser. A rat extracellular matrix and adhesion molecule microarray was used to determine genes modulated by PDT. Adhesion to collagen IV following PDT was evaluated and immunohistochemical analysis of PDT-treated orthotopic rat prostate tumors was performed. Finally we used an *in vivo* flow cytometer to determine the effect of PDT on circulation of prostate tumor cells.

Out of 111 genes on the microarray no gene was upregulated by PDT treatment. However, 32 genes were downregulated more than 2-fold, and 9 genes were downregulated more than 4 fold. The most inhibited gene was Integrin α5 (7.5 fold), and western blot confirmed this downregulation. Functional analysis of integrin α5β1 showed a decrease in adhesion to the extracellular matrix protein collagen IV (10% of control) 24h after PDT, but normal adhesion 72h after PDT. *In vivo* circulation of PDT treated prostate tumor cells showed an increase in circulation time 24h after treatment but normal circulation time 72h after treatment. PDT treated orthotopic tumors showed a decrease in integrin α5β1 levels.

The results presented here establish that PDT of prostate cancer decreases expression of integrin α5β1 and increases circulation time of prostate cancer cells. We have previously shown that combination of PDT with anti-angiogenic treatment increases survival. Together, these observations suggest that inadequate PDT may have long-term effects that may detract from its excellent local control.

OC34

Modulation of cellular Ca²⁺ signalling during PDT

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During photodynamic therapy (PDT) the interaction between light and photosensitizers induces oxidative stress and the production of reactive oxygen species (ROS). ROS formation influences ion channels and causes changes in the Ca²⁺ concentration which may induce further reactions leading to cell stimulation or cell death. The aim of our study was to analyse the intracellular production of ROS and the correlation with spatial and temporal changes of Ca²⁺ in dependence on photosensitizer localization and irradiation conditions.

In order to analyse subcellular Ca²⁺ signalling during PDT Fluo3 was used as fluorescence reporter. In addition, rat epithelial cells (RR 1022) and U373 glioblastoma cells were stably transfected with Ca²⁺ sensitive vectors. One of the vectors code for a fusion protein of calmodulin and the GFP-derivative cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) (cameleon vector). To measure a mitochondrial Ca²⁺ signal we used the so called camgaroo vector, consisting on calmodulin and YFP which was subcloned into a mitochondria-specific expression vector. ROS were induced with two different photosensitizers (hypericin and AlPcS₄). Ca²⁺ signalling was observed by laser scanning microscopy (LSM). For spectral imaging the LSM510 Meta system was used (Carl Zeiss).

Low dose PDT induced Ca²⁺ oscillations, which might be induced by Ca²⁺ shuttling processes between mitochondria and ER. With higher doses, Ca²⁺ overload and subsequent Ca²⁺ release was observed. In correlation with this dose blebbing of the cells could be detected, indicating apoptosis. Whereas U373 glioblastoma cells responded even at low PDT doses, the threshold for RR1022 cells was significantly higher.

Oxidative stress during PDT induces modulation of cellular Ca²⁺ which reflects Ca²⁺ shuttling between organelles or otherwise Ca²⁺

overload, depending on drug/light dose. With low dose PDT the cells seemed to be activated, whereas at higher doses cell death occurred.

OC35

Photoeffects of zinc(II)-phthalocyanine on cell-substrate adhesion in mouse keratinocytes

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The disruption of cell attachment is a process that results in the activation of apoptosis by anoikis. It has been described that the increase of cell adhesion to the substrate is related with resistance to anoikis in tumoral cells. However, the implication of adhesion to substrate in the induction or resistance to cell death by photosensitizers (PSs) is unclear. We have studied the combined effects of zinc(II)-phthalocyanine (ZnPc) and red light on murine keratinocytes (Pam-212) and on these cells transfected with retroviruses encoding V12H-Ras (Pam-Ras). In particular, we have analyzed the photoeffects on cell survival, cell-substrate adhesion, and its relation with apoptosis in both Pam-212 and Pam-Ras cells. The treatment conditions were selected from uptake experiments (2 h of incubation accumulates approximately 30 nmoles of PS/mg of protein in both cell types), and they consisted in 2 h incubation with 5×10^{-7} M ZnPc followed by 4 min red light irradiation. The photodynamic treatment (PDT) resulted in an increase of cell death by apoptosis in both cell types. However, Pam-Ras cells were more resistant to photodynamic treatment than Pam-212 (around 60% vs. 50% lethality in Pam-212 and Pam-Ras, respectively). In addition, Western blot analysis showed a higher expression of both β_1 -integrin and phosphorylated FAK in Pam-Ras cells during the first hours after treatment when compared to Pam-212 cells. Conversely, the activation of caspase 3 and the degradation of PARP and β -catenin occurred earlier in Pam-212 than in Pam-Ras cells. All these results indicate that these experimental treatments induce an increase of cell-substrate adhesion in keratinocytes overexpressing the *ras* oncogene and this feature could be implicated in their resistance to cell death by apoptosis.

OC36

Photodynamic injury of isolated crayfish stretch receptor: death of glial cells and neuroglial interactions

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Photodynamic therapy is used for treatment of cancer including brain tumours. However, PDT injury of normal glial cells and neuroglial relationships occurring under PDT impact are not explored. We used a simple model system, isolated crayfish mechanoreceptor, consisting of two sensory neurons enveloped by satellite glial cells. Neuron functional state was monitored electrophysiologically. Double fluorochroming of this preparation with Hoechst 33342 and propidium iodide allowed visualization of alive, necrotic and apoptotic cells. PDT effect of Photosens (AIPcS_n) inhibited and then irreversibly abolished neuron activity. In the next 8 h percent of necrotic glial cells progressively increased. Apoptosis of glial cells became significant 6-8 h after the treatment. Neuron nuclei progressively shrank, but their apoptotic fragmentation did not occur. PDT-induced death of some glial cells was accompanied by increase in their total number. Preliminary neuron inactivation by a laser beam focused to its body increased percent of apoptotic but not necrotic glial cells. Therefore, the neuron supports survival of surrounding glial cells. Inhibition of adenylate cyclase by MDL-12330A or tyrosine phosphatase by sodium orthovanadate protected glial cells from PDT-induced necrosis but not apoptosis. In contrast, adenylate cyclase activation by forskolin enhanced PDT-induced apoptosis of glial cells. Inhibition of tyrosine kinase by genistein did not affect necrosis or

apoptosis of glial cells. These data show involvement of adenylate cyclase and tyrosine phosphatase signalling pathways in PDT-induced death of glial cells.

The work was supported by RFBR grants 02-04-48027, 05-04-48440a.

OC37

Cell specific effects of polyunsaturated fatty acids on 5-aminolevulinic acid based photosensitization

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Objective: the purpose of this study was to examine whether the dietary components n-6 and n-3 polyunsaturated fatty acids (PUFAs) may potentiate the effect of photodynamic therapy (PDT) in human cancer cell lines by enhancing lipid peroxidation.

Methods: the effects of the porphyrin precursor 5-aminolevulinic acid (5-ALA) and light ($320 < \lambda < 440$ nm, 33 W/m²), with or without docosahexaenoic acid (DHA) or arachidonic acid (AA), were tested in the colon carcinoma cell lines SW480 and WiDr, the glioblastoma cell line A-172 and the lung adenocarcinoma cell line A-427.

Results: the production of endogenous protoporphyrin IX (PpIX) and the cell killing by 5-ALA-PDT varied substantially between the cell lines, but without clear correlation with PpIX levels. Treatment with DHA or AA (10 or 70 μ M, 48 or 72 h) in combination with 5-ALA-PDT (1 or 2 mM) enhanced the cytotoxic effect in A-172 and A-427 cells, but not in SW480 and WiDr cells. While 5-ALA-PDT alone increased lipid peroxidation in A-172 and WiDr cells only, 5-ALA-PDT plus PUFAs increased lipid peroxidation substantially in all four cell lines. Interestingly, α -tocopherol (50 μ M, 48 h) strongly reduced lipid peroxidation after all treatments in all cell lines, while cytotoxicity was only reduced substantially in A-427 cells.

Conclusion: this demonstrates that induction of lipid peroxidation is not a general mechanism responsible for the cytotoxicity of 5-ALA-PDT, although it may be important in cell lines with an inherent sensitivity to lipid peroxidation products. Thus, the mechanisms of cell growth inhibition/cell killing by PDT are complex and cell specific.

OC38

Detecting apoptosis after PDT: PET imaging with Cu-64 labelled streptavidin following pretargeting of phosphatidylserine with biotinylated annexin-V

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Tumor regression after photodynamic therapy (PDT) may result from different action mechanisms, including the induction of apoptosis. A priori, radiolabeled annexin-V is an ideal probe for *in vivo* apoptosis detection, due to its strong affinity for the apoptotic marker phosphatidylserine, the molecular flag on the surface of apoptotic cells. Here, tumor apoptosis after therapy is clearly visualized by positron emission tomography (PET) using a three-step procedure. Apoptotic cells are pretargeted with biotinylated annexin-V, followed by an avidin-chase to reduce levels of free biotin. Apoptosis is then detected with ⁶⁴Cu-labeled streptavidin. Apoptosis was induced in EMT-6 tumor-bearing Balb/c mice by PDT using disulfonated metallo phthalocyanine dyes (AIPcS₂ or ZnPcS₂) as photosensitizers and 680 nm laser light. Externalized

phosphatidylserine was pretargeted with biotinylated annexin-V and subsequently visualized with a ^{64}Cu -labeled streptavidin complex (^{64}Cu -DOTA-biotin-SAV). The latter was prepared by loading streptavidin with ^{64}Cu -DOTA-biotin. Mice were injected iv with biotinylated annexin-V at different time intervals following PDT. Two hours later endogenous biotin was removed with an avidin-chase and after another 2 h ^{64}Cu -DOTA-biotin-SAV was administered. PET images were subsequently registered from 0.5-9 h. Using conventional staining methods, we confirmed that apoptosis occurred as early as 3 h post-PDT and was detectable up to 24 h post-PDT. PET images clearly delineate apoptosis in treated tumors as early as 30 min after ^{64}Cu -DOTA-biotin-SAV administration with tumor-to-background ratios reaching a maximum at 3 h post-injection. In addition to the extent of apoptosis, differences in tracer uptake patterns reveal differences in action mechanisms between the photosensitizers. Our data demonstrate the efficacy of a three-step ^{64}Cu -pretargeting procedure for PET imaging of apoptosis and confirm the usefulness of small animal PET to evaluate cancer treatment protocols.

OC39

Intracellular trafficking of a photosensitizer immunoconjugate using quantum dots: a technique for selective photodynamic therapy

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The overall goal of this study is to develop a selective approach for the PDT of residual disease in advanced ovarian cancer. Photoimmunotherapy (PIT) is a targeting strategy that involves the activation by light of a photosensitizer (PS) conjugated to a monoclonal antibody (Mab) to produce a photosensitizer immunoconjugate (PIC). We have developed a PIC of C225 with the PS, benzoporphyrin derivative (BPD). C225, a chimeric Mab blocks the Epidermal Growth Factor Receptor (EGFR) which is overexpressed in ovarian cancer cells and is vital for tumor growth, survival and spread. The combination of PDT and C225 introduces a dual enhancement to cancer therapy: (1) improved cytotoxicity over the antibody alone and, (2) enhanced selectivity compared to the PS (BPD) alone. Using quantum dots and organelle trackers, PIC binding and intracellular trafficking in ovarian cancer cells (OVCAR-5) is established. We show that the PIC binds to the cell membrane at early time points (<5 min). At longer incubations lysosomal localization of the PIC is observed and SDS-PAGE analyses of cell extracts established the processing of the PIC. Application of low light doses at these times showed relocalization of the fluorescence from the lysosomes to extra-lysosomal sites. We also show that the uptake, phototoxicity, localization kinetics and long-term survival in ovarian cancer cells are different for the unconjugated and the conjugated BPD. Taken together our data suggest that PIT provides (1) a selective modality for killing of ovarian cancer cells and, (2) relocalization of the PIC maybe useful for enhancing PIT efficacy. Additionally, we are investigating the application of quantum dots directly conjugated to C225 to detect tumor burden for *in vivo* assessment of the therapeutic efficacy. Majority of the women present the advanced form of the disease with a bleak 5-yr survival rate of 21%. If successful, PIT could be a promising option for women diagnosed with advanced ovarian cancer.

OC40

Synthesis and *in vitro* testing of photosensitizer-peptide conjugates for use in photodynamic therapy

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One barrier to the widespread success of Photodynamic Therapy has been the relatively poor tumour selectivity demonstrated by

current systemically administered photosensitizers. This problem can manifest as skin photosensitivity or normal tissue damage following exposure to activating light. Many new photosensitizers have been developed worldwide but the focus has generally been on the improvement of the photo-physical properties such as the wavelength of absorption, extinction coefficients and singlet oxygen quantum yield. Although these properties are important they have little influence on the selectivity of the photosensitizers for tumour tissue.

We have used a solid phase peptide synthetic chemistry approach to produce peptide-photosensitizer conjugates which differ in either their targeting peptide or photosensitizer. One such peptide contains the $\alpha_v\beta_3$ integrin binding motif -arg-gly-asp- (RGD). This integrin is exclusively found in tumour neovasculature and is a potential target for cancer therapies. We have compared the ability of linear, cyclic, random and reverse orientations of conjugates containing this sequence to binding to the specific integrin.

A number of photosensitizers were studied for their ability to efficiently conjugate with peptides. Protoporphyrin IX, purpurin 18, a phenothiazine and two phthalocyanine derivatives have all been tested. Conjugation of the phthalocyanines and phenothiazine proved unsuccessful for different reasons but protoporphyrin and purpurin-18 were excellent candidates.

We have demonstrated *in vitro* that the a cyclic RGD containing peptide-photosensitizer conjugate is not only a good photosensitising agent but also maintains its integrin binding specificity. In $\alpha_v\beta_3$ integrin expressing cells the levels of associated sensitizer was higher after incubation with conjugate compared to free sensitizer.

Development of this approach to photosensitizer targeting may greatly improve *in vivo* photosensitizer selectivity.

IL41

P53 mutations as early events in UV carcinogenesis

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The appearance of non-melanoma skin cancer is a complexly regulated multistep process. UVB irradiation is the main inducer of these tumors despite of several cell and tissue level defense mechanisms that include, among others, the overview of cell-cycle and apoptosis regulation. A central player in this extensively guarded regulation network is the tumor suppressor gene product *p53*. It has been shown that *p53* acts at various levels in the war against cancer; therefore, its inactivation is a key event in cancerous progression of UV-hit keratinocytes. Late stages of tumors are known to frequently harbor *p53* mutations, yet limited data were available from early steps on cancer developments. In this review further insight is given on the latest findings in various experimental settings that were aimed to reconstruct the development of human skin cancers in a murine experimental system. Also, non-tumor bearing human skin analysis data point to the direction that *p53* mutations are crucial from a single cell stage for non-melanoma skin cancer formation. Taken together, these data show convincing evidence that the role of UVB goes beyond what was previously expected by inducing, promoting and maintaining tumor growth and *p53* inactivation is a rate-determining step in this process.

IL42

UV-related p53 mutations in human skin carcinoma and precursor stages

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Skin cancer develops as a consequence of multiple, irreversible genetic alterations in a susceptible cell. Ultraviolet radiation (UVR) is accepted as the major cause of skin cancer, and alterations of the

p53 tumor suppressor gene are very common in non-melanoma skin cancer. The mutations predominantly affect adjacent pyrimidines consistent with UVB playing a causative role. The high frequency of p53 mutations in chronically sun-exposed skin, actinic keratosis (AK), squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) indicates that alteration of the p53 gene is a key and perhaps the initiating event in skin carcinogenesis. Skin provides an advantageous model for studying the development of cancer. Detectable lesions occur early during tumor progression, facilitating molecular analysis of cell populations from both preneoplastic and neoplastic lesions. The abundance of clones containing p53 mutated keratinocytes adjacent to BCC and SCC indicate a role in skin carcinogenesis. Advanced laser-assisted microdissection of cells defined under the microscope ensures a selection of representative material without contamination by neighboring cells. By using well optimized molecular methods for genetic analysis it is possible to read the entire p53 gene sequence in minute cell samples or even in single cells. Studies using p53 mutations as a clonality marker suggest a direct link between actinic keratosis, SCC *in situ* and invasive SCC. BCCs often consist of subclones and it has been shown that different parts of a tumor share a common p53 mutation but differ with respect to a second, third or even a fourth mutation within the p53 gene. Here we present examples of using well-defined cell populations, including single cells, from complex tissue in combination with molecular tools to study the multi-hit history of a tumor by exploring the remnants of genetic events.

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IL43

Inverse relationship between increased apoptosis and decreased skin cancer in UV-irradiated *CD1d*^{-/-} mice

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CD1 is a nonpolymorphic major histocompatibility complex I-like molecule. Murine CD1 has been implicated in the development and function of Natural Killer T cells. Previous studies demonstrated that *CD1*^{-/-} mice are resistant to UV-induced immune suppression, skin damage, and skin cancer development compared to wild-type mice. To determine the mechanisms involved, we investigated the time course and kinetics of keratinocyte cell death after UV irradiation. In acute UV experiments (5 kJ/m²), the number of TUNEL-positive keratinocytes peaked at 24-48 h after irradiation in both groups. However, the TUNEL-positive keratinocytes were eliminated from the skin at 72 h post-UV in WT mice, but they still persisted until 96 h post-UV in *CD1*^{-/-} mice. The kinetics of p53 expression closely followed the kinetics of apoptotic cell death. The persistence of apoptotic keratinocytes in *CD1*^{-/-} mouse skin correlated with high levels of p53 expression at 96 h post-UV. Chronic UV irradiation for 1-3 wk resulted in induction of a significantly higher number of apoptotic keratinocytes in *CD1*^{-/-} mice than in WT mice. After 12 wk of chronic UV, clusters of p53-positive, abnormal keratinocytes were present in WT mouse skin, but not in *CD1*^{-/-} mouse skin. In addition, epidermis and dermis taken from chronically UV-irradiated *CD1d*^{-/-} mice harbored significantly fewer p53 mutations than WT mice. These results indicate that the resistance of *CD1*^{-/-} mice to UV-induced skin damage and UV carcinogenesis may in part be due to increased cell death and elimination of keratinocytes containing DNA damage and p53 mutations.

IL44

Effect of *DDB2* (Damaged DNA Binding) gene dose on sensitivity to UV carcinogenesis in hairless mice

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Xeroderma pigmentosum E patients have an elevated risk on skin tumors due to reduced DNA repair. These patients bear mutations in the *DDB2* gene, resulting in a loss of p48 activity. The p48 protein is assumed to play a role in recognition of UV-induced photo lesions in global genome nucleotide excision repair (GG NER). In contrast to humans, mice are deficient in GG NER of cyclo-butane pyrimidine dimers (CPDs), and have a very low expression of p48 in the skin. Therefore one would expect a delayed development of skin tumors in *DDB2*-transfected mice under chronic UV exposure, and an accelerated development in *DDB2*-knockout (KO) mice.

To test this hypothesis hairless *DDB2*-transfected and wild-type (WT) littermates were irradiated with 1 Minimal Erythema/edema Dose (MED) UV (500 J/m² from TL-12 lamps) per day. *DDB2*-KO, *DDB2* heterozygous KO and WT littermates were irradiated with 0.5 MED UV/day. Visual scoring on skin tumor type, size and numbers was performed weekly.

DDB2-transgenic mice developed squamous cell carcinomas (SCCs) later than WT littermates: the median tumor induction time (t₅₀) was 90 (85-96, 95%CI) days, while the t₅₀ for WTs was 78 (74-84, 95%CI) days. *DDB2*-KO mice revealed acceleration of UV carcinogenesis: the t₅₀ of homozygous knockout being 63 (57-70, 95%CI) days, in heterozygous knockout 76 (68-84, 95%CI) and in WT littermates 100 (88-114, 95%CI) days.

We can state that there is a dose-effect relation between *DDB2* gene expression levels and sensitivity to UV carcinogenesis. Recent experiments support the hypothesis that *DDB2* protects mice against UV-carcinogenesis by increasing GG NER of photo lesions in the epidermis.

OC45

Oncogenic potential of melanoma associated mutant BRAF

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Melanoma represents the fastest growing malignancy in the western world. Several studies, including ours, have shown that mutations in *BRAF* and *RAS* genes are mutually exclusive in human melanomas and do not correlate with the metastatic potential. Expression analysis of the BRAF-MEK1-ERK1/2 signaling pathway showed no differences in the expression of BRAF, but the downstream ERK1/2 MAPK was activated at higher levels with increasing metastatic potential. Because some human melanomas harbor BRAF mutations whereas others harbor RAS mutations, it is important to determine their relative oncogenic potential. To study the oncogenic potential of mutant BRAF, we generated a *BRAF*^{V600E} expression vector by site-directed mutagenesis. We transfected mouse embryo fibroblasts (MEFs) that lack p53 (p53^{-/-}) or contain homozygous mutations at codon 172 (R172H and R172P) with *BRAF*^{V600E} cDNA or with plasmid DNA containing mutant *H-RAS*^{G12V} and determined their ability to induce morphologically transformed foci in reduced serum medium. Results indicated that both *BRAF*^{V600E} and *H-RAS*^{G12V} induced morphologically transformed foci in p53^{-/-} and p53^{R172H} MEFs, but not in p53^{R172P}. Interestingly, *BRAF*^{V600E} was less potent than mutant *H-RAS*^{G12V} in cooperating with mutant p53 in focus formation assays. The number and size of foci induced by *BRAF*^{V600E} was significantly fewer and smaller than the foci

induced by mutant *H-RAS*^{G12V}. Transformed clones were isolated, expanded, and expression of *BRAF*^{V600E} or *H-RAS*^{G12V} was verified. *In vitro* growth characteristics and *in vivo* tumorigenesis corroborate the transformed phenotype, but *H-RAS*^{G12V} transformed cells demonstrated an increased growth over those transformed with the *BRAF*^{V600E}. Thus, these results indicate that mutant *BRAF*^{V600E} is weakly oncogenic compared to mutant *H-RAS*^{G12V} and that they both cooperate with *p53*^{-/-} and *p53*^{R172H}, but not with *p53*^{R172P} in oncogenic transformation.

OC46

Molecular analysis of risk factors associated with skin cancers from immunosuppressed renal transplantation patients

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Immunosuppressed renal transplant recipients (RTR) present a higher incidence of non-melanoma skin cancers (NMSC), predominantly squamous cell carcinomas (SCC), than that found in the general population. To understand their high skin cancer susceptibility we have analyzed *p53* mutation and expression in RTR skin lesions in relation to *p53* codon-72 genotype and Human Papilloma Virus (HPV) status. We also investigated whether skin cancer predisposition in RTRs was associated with XPD gene polymorphisms. Among 38 skin lesions, 24 *p53* mutations were detected in 60% of the NMSC and in 41% of the precancerous actinic keratosis (AK), suggesting that *p53* mutations are early events in the skin carcinogenesis process of RTRs. Immunohistochemical analysis shows good correlation between *p53* accumulation and mutations in the NMSC. Among the 24 *p53* mutations in NMSC, one was a deletion and 23 were base substitutions, the majority (78%) being the UV specific C to T transitions located at bipyrimidine sites. Interestingly, 35% (6/17) were tandem mutations, 4 being the UV signature CC to TT transitions. We found no significant differences in the *p53* codon 72 or XPD genotype frequency of our RTRs compared with control populations of the same ethnic group. HPV DNA was detected in 78% of the RTR tumors which is significantly higher than that found (26-53%) in skin cancers from the normal population. Moreover, the epidermodysplasia verruciformis HPV, linked to SCC development, is the predominant type found in the RTR skin tumors. We do not find an association between HPV status and *p53* mutation. These data indicate that *p53* codon-72 or XPD gene polymorphisms are not involved in skin cancer susceptibility of RTRs. Our study is the first to show the high level of UV hallmark tandem mutations in NMSC of RTRs together with other UV specific *p53* mutations indicating the major role of the UV component of sunlight in the skin carcinogenesis of RTRs.

OC47

Loss of expression of CDKN2A via deletion and promoter hypermethylation in human non melanoma skin cancers

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The *CDKN2A* locus at human chromosome 9p21 encodes two alternative reading frame proteins (p16INK4a and p14ARF) known to function as tumor suppressors via the retinoblastoma or *p53* pathway. Inactivation of *CDKN2A* can lead to deregulation of these two pathways. Although mutations in the *p53* gene are uncommon in human melanoma, loss of the tumor suppressor activity of *CDKN2A* in human familial and sporadic melanoma is well documented. In addition, inactivation of *CDKN2A* involving mutations, deletions or promoter methylation has been found in a variety of human tumors. However, it is not clear whether genetic

alterations in the *CDKN2A* play a role in the development of human non melanoma skin cancer (NMSC). We, therefore, analyzed 40 NMSC (21 primary human squamous cell carcinomas, 17 basal cell carcinomas, 17 basal cell carcinomas and 2 actinic keratoses) for mutations in exons 1 alpha, 1 beta and 2 of the *CDKN2A* locus using PCR and SSCP techniques. The results indicated that none of the tumors contained mutations in the *CDKN2A* gene. However, immunohistochemical analysis revealed loss of expression of p16 and p14 proteins in 97% of NMSC, suggesting that hypermethylation of the promoter region may be responsible for the silencing of these genes. In fact, methylation specific PCR experiments revealed that about 45% of tumors had hypermethylation of *CDKN2A* in the promoter regions. As expected, about 80% of NMSC contained UV signature mutations in the *p53* gene and about 90% of the tumors were strongly positive for *p53* immunostaining. Based on these data, we conclude that in addition to the mutations in the *p53* gene, silencing of *CDKN2A* gene expression via promoter hypermethylation may play an important role in human NMSC development.

OC48

The increase in melanoma: are dietary photosensitizers responsible?

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There are three known causes of melanoma: (1) genetic predisposition including xeroderma pigmentosum, (2) use of oral 8-Methoxy Psoralen in PUVA therapy of psoriasis, and (3) use of topical or oral 5-Methoxy Psoralen in suntanning products and in PUVA therapy of psoriasis. The incidence of melanoma has been increasing at a constant rate in North America and in northern Europe since before WWII. This makes periodic changes in UV exposure habits less than a compelling explanation. Psoralens are present in many food substances, including citrus, celery, parsley, cilantro, carrots and parsnips. It is estimated that the average US Caucasian diet contains over 1 mg of psoralen each day. Psoralens are not altered by cooking. A comparison of the fivefold rate of increase in melanoma for almost 50 years to the fivefold increase in the rate of citrus consumption is more than suggestive of a causal relationship. Melanoma incidence has recently been correlated with orange juice consumption ($p \leq 0.008$) in a large study of nurses. The simple fact that the more affluent, diet conscious, individuals historically are at greater risk to melanoma is also suggestive.

IL49

The Photosystem II reaction centre of *Acaryochloris marina*, a predominantly chlorophyll *d* containing cyanobacterium

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The characteristics of photosystem II, isolated from a novel cyanobacterium, *Acaryochloris marina*, have been studied. *A. marina* has as its major pigment, chlorophyll (Chl) *d* which absorbs 30 nm further to the red than Chl *a*. Surprisingly a low level of Chl *a* is always found in this organism: even when it is grown at very low light intensities cells have been reported still to have 2 Chl *a* per PS II reaction centre. The excited state energy provided by Chl *d* is estimated to be at least 100 mV less than that found for Chl *a*. It has, therefore, been suggested that the special pair of Chls, bound to the D1 and D2 proteins at the heart of PS II, are actually molecules of Chl *a*. So far no sufficiently purified PS II particles have been isolated to address this problem and the nature of the pigments which comprise the primary electron donor in PS II is still

under debate. Here, we report the isolation of PS II enriched preparations which have less than 1 Chl *a* per PS II reaction centre and we conclude that Chl *d* rather than Chl *a* drives the primary electron transfer reactions in PS II. This conclusion has very interesting consequences for the study of the energetics of charge separation in oxygenic photosynthetic organisms.

IL50

Biomimicking photochemical reactions in artificial photosynthetic complexes

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Keywords: artificial photosynthesis, manganese, ruthenium, charge separation, EPR

Our work aims at utilizing solar energy for fuel production, via a molecular system for artificial photosynthesis. In photosynthesis, Photosystem II (PSII) uses sunlight as driving force to extract electrons from water. To mimic essential structural and functional parts of the water oxidizing complex (WOC) in PSII, we have synthesized several multinuclear ruthenium-manganese complexes, that undergo light-induced electron transfer from Mn to Ru.

In our systems we use ruthenium^{II}-trisbipyridine (Ru^{II}(bpy)₃) complexes as photosensitizer, to mimic the function of P₆₈₀. We have demonstrated that a dinuclear Mn complex, covalently linked to Ru^{II}(bpy)₃, could be oxidized several steps from Mn₂^{II,II} to Mn₂^{III,IV} via light-induced electron transfer, in the presence of an exogenous electron acceptor. In order to increase the similarity to the water oxidizing cluster, we have designed a series of dinuclear Mn complexes with different N/O ratios. These can form five separate oxidation states within a similar redox potential range as that of water oxidation.

Recently, we synthesized a donor-photosensitizer-acceptor triad molecule, where a Ru^{II}(bpy)₃ was covalently linked to two naphthalene diimide (NDI) molecules, to function as electron acceptor, and to a Mn₂^{II,III}(bpmp)(OAc)₂ complex, as electron donor. This is the first synthetically linked electron donor-sensitizer-acceptor triad in which a manganese complex plays the role of the donor. EPR spectroscopy was used to demonstrate the light induced oxidation of the manganese dimer (Mn₂^{II,III}(bpmp)(OAc)₂), and reduction of the naphthalenediimide (NDI^{•+}) acceptor moieties, while optical spectroscopy was used to follow the kinetic evolution of the NDI^{•+} radical.

The charge-separated state is very long-lived, with an average lifetime of the NDI^{•+} radical of ~ 600 μs at room temperature, which is at least two orders of magnitude longer than for previously reported triads based on a [Ru(bpy)₃]²⁺ photosensitizer. At 140 K, this intra-molecular recombination was slowed down, with a lifetime of 0.1-1 s. The long lifetime is explained by an unusually large reorganization energy. This makes the reaction strongly activated despite the large driving force (-ΔG⁰ = 1.03 eV). Following the primary charge separation, we could observe oxidation of the linked Mn complex.

These results suggest that the molecular triad is capable of undergoing photoinduced intramolecular electron transfer, leading to a long lived charge-separated state similar to the electron transfer chain in the donor side of natural photosystem-II.

IL51

Biomimetic models of Photosystem II driven by ruthenium complexes

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Despite the degree of sophistication, which can now be incorporated into biomimetic ligand design, there is a growing awareness amongst bioinorganic chemists of the need to incorporate into their model complexes mimics of not only immediate metal coordination sphere, but also mimics of other features of the active site. The chemistry we will discuss concerns the biomimetic model for the first electron transfer in PSII. In the recently published X-ray structure of PSII (Barber et al. Science 2004) it is shown that the TyrZ-His190 pair is not directly involved in the coordination sphere of the manganese cluster. Even if the role of this pair of amino acids is unclear, in their absence the PSII is inactive. Hence with these issues in mind we have developed a family of phenanthroline based ligand holding an imidazole and a phenol rings. In one of the molecules described this pair is in hydrogen bonding interaction as in the natural system. The metallocomplexes of the type [Ru(bpy)₃]²⁺ are characterised and the combined photophysical and spectroelectrochemical behaviours will be exposed. Our collected data evidenced the photogeneration of a phenoxyl radical when the Ruthenium complex was irradiated in the presence of an external electron acceptor, therefore mimicking the first electron trade between P₆₈₀⁺ and TyrZ-His190. Density Functionnal Theory and Time Dependent-DFT calculations were performed to support our experimental data.

IL52

Bioinspired energy conversion schemes

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A photoelectrochemical cell that uses enzyme-assisted oxidation to reform carbohydrates, alcohols and other biological materials to hydrogen has been developed. The photoanode consists of a Grätzel-type nanoparticulate TiO₂ electrode coated with a porphyrin sensitizer. Upon visible light excitation, electrons are injected from the S₁ state of the porphyrin into the TiO₂ conduction band. This produces highly reducing electrons at ~ -0.5 V (vs. SHE) and highly oxidizing porphyrin radical cations at ~ 1.23 V (vs. SHE). Electrons at the negative potential are then passed through an external circuit to a microporous platinum cathode where hydrogen evolution occurs with a quantum yield of up to ~ 5%.

NADH is oxidized by the porphyrin radical cation to NAD⁺, which regenerates the porphyrin ground state for subsequent rounds of photo-excitation and poises the NAD⁺/NADH coenzyme couple oxidizing. Glucose, ethanol or other reduced carbon components of biomass in the anode solution are oxidized by the appropriate NAD-linked dehydrogenase enzyme(s), restoring NAD⁺ to NADH. Under modest illumination (~ 1 mW/cm²) and using ethanol and alcohol dehydrogenase, the cell maintains more than 200 mV differential redox potential between the cathode and anode compartments. The key to successful operation of the cell is the design of a porphyrin sensitizer that is capable of excited state electron injection into the TiO₂ CB, is able to smoothly oxidize NADH to NAD⁺ without damaging it, and is sufficiently oxidizing to poise the NAD⁺/NADH couple oxidizing.

IL53**The photochemistry of novel flavin-based photoreceptors**

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The flavoproteins AppA from *Rhodobacter sphaeroides*, Slr from *Synechocystis* PCC 6803 and YcgF from *E. coli* contain an N-terminal domain belonging to a new class of flavin-binding blue-light photoreceptors designated as BLUF (Blue-Light sensing Using Flavin) domains. AppA was shown to control photosynthesis gene expression in response to blue light and oxygen tension, whereas Slr is involved in the phototaxis of *Synechocystis*. BLUF domains are characterized by a long-lived signaling state that exhibits a 10 nm red-shift of the oxidized flavin absorption bands, and is thought to arise from a hydrogen-bond rearrangement between the flavin and the residues lining the binding pocket. We have investigated the photochemistry of these new photoreceptors by ultrafast fluorescence and femtosecond transient absorption spectroscopy. Time-resolved fluorescence experiments on AppA revealed a multiexponential decay of excited flavin adenine dinucleotide (FAD), with lifetimes ranging from 100 ps up to 3 ns. Transient absorption spectroscopy revealed a similar multiexponential decay of excited FAD, with time constants of 90 ps, 590 ps and 3 ns. Concomitant with the decay of excited FAD, the rise of the 10 nm red-shifted signaling state of AppA was detected. Thus, AppA has a unique photocycle whereby the signaling state is formed on the ultrafast timescale directly from the FAD singlet excited-state, and remains stable over 12 decades of time, i.e. from < 1 ns to half an hour. In parallel with the signaling state, the FAD triplet state is formed as a side reaction of the AppA photocycle. In *Synechocystis* Slr and *E. coli* YcgF, the FAD excited-state lifetime is much shorter than in AppA, 5 ps and 11 ps respectively, and proceeds through an as of yet unidentified intermediate to the signaling state in about 150 ps. The implications for the reaction mechanism will be discussed in the light of the recently determined crystal structure of AppA.

IL54**Effects of ultraviolet on vision and the human eye—acute and delayed**

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Ultraviolet radiation is largely absorbed in the anterior structures of the eye, but the exact site of absorption varies with wavelength. UV-C is superficially absorbed in the cornea and can produce photokeratitis. UV-B is largely absorbed in the cornea, but the small fraction that reaches the lens has been shown to produce acute cataracts in experimental animal models, and some epidemiological studies point to UV-B as a causal factor in age-related cortical cataract. UV-A is primarily absorbed in the lens and produces fluorescence. Several theories propose that UV-A also contributes to the risk of cataracts from chronic exposure, but evidence from epidemiological studies is weak. The human retina is exposed to UV-A and even some longer UV-B wavelengths in early childhood, but normal aging of the lens is reflected by “yellowing” with far greater attenuation of UV reaching the retina. Less than one percent of incident UV-A reaches the retina in a young adult. The differential spectral exposure of ocular structures result from the series of intra-ocular spectral filters (cornea, aqueous, lens, vitreous) which each tend to filter more and more of the shortest wavelengths. The differential geometrical exposure of each ocular structure depends upon both behavioral and anatomical factors. A person generally avoids direct viewing of the sun. The anatomical shielding by the upper lid is not always recognized but accounts for the single-most important factor in limiting excessive exposure in many environments.

IL55**Effects of sunlight on the human eye – the ophthalmohelioses**

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The ophthalmohelioses are sunlight related eye diseases involving the eyelids, cornea, conjunctiva, crystalline lens, uvea and retina. It has become increasingly apparent that the effects of sunlight are much more insidious and detrimental to the eye and vision than had been suspected previously. The effects may be acute (usually after a latent period), long term after an acute exposure, or chronic following long-term exposure to levels of UV below those required for acute effects. Human studies have been confined to the cornea and conjunctiva, specifically to develop action spectra for acute exposures (photokeratitis and photoconjunctivitis). The action spectrum for the lens was derived using animal models, *in vivo* and tissue culture protocols. In order to study the chronic effects of sunlight exposure it is necessary to compare large populations of matched individuals with varying lifetime exposures to sunlight. These epidemiologic studies lack accurate dosimetry and are confounded by numerous other risk factors. However, they do implicate solar ultraviolet (mainly UV-B) as a causal or contributing factor for the development of pterygium, climatic droplet keratopathy, and some forms of human age related cataract. Although direct viewing of the sun produces photoretinitis (e.g. “eclipse blindness”) by both thermal and photochemical mechanisms, the role sunlight plays in the development of age related macular degeneration is less clear.

IL56**Production and clearance of all-trans retinol in bleached rod and cone photoreceptors***P. Ala-Laurila¹, M. Estevez¹, R.K. Crouch², Y. Koutalos², B. Wiggert³, M.C. Cornwall¹**¹Department of Physiology and Biophysics, Boston University School of Medicine, Boston, MA; ²Department of Ophthalmology, Medical University of South Carolina, Charleston, SC; ³National Eye Institute, National Institutes of Health, Bethesda, MD, USA*

The purpose was to determine the effects of photoreceptor morphology and visual pigment type on the all-trans retinal to all-trans retinol reduction and the clearance of retinol from the cell after bleaching.

Measurements were carried out on isolated red and blue cones, and red and green rods of the tiger salamander and on the rods of the Tokay gecko. The spatial and temporal distribution of all-trans retinol was measured by microfluorometry after a bright light exposure bleaching the visual pigment. Simultaneous electrophysiological recordings were performed to identify the photoreceptor types. Cells were treated with interphotoreceptor retinoid binding protein (IRBP, 100 μ M) to determine its effect on retinol kinetics.

All-trans retinol is produced over an order of magnitude faster in salamander cones than in red rods. In both gecko rods and salamander green rods, which contain cone-type visual pigments in a rod-type morphology, the production of all-trans retinol is *ca.* 3-5 times faster than in salamander red rods with rod-type pigment in a rod-type morphology. Green rods and blue cones have been shown previously to contain identical pigments, which resides in internal disks in green rods and in the plasma membrane in blue cones. The rate of retinol production and clearance are significantly faster in blue cones than in green rods. In general, the clearance of all-trans retinol is *ca.* 7-26 times faster in the photoreceptors with cone-type morphology than in the photoreceptors with rod-type morphology. IRBP significantly facilitates the clearance of retinol from both rods and cones but affects only little the rate of retinol production. We suggest a model in which the rate of the reduction of all-trans retinal to all-trans retinol is defined by the visual pigment type as well as the availability of the reducing agent. Retinol clearance can be modeled as a simple diffusion process, the rate of which is defined by the photoreceptor morphology.

IL57**The role of the retinoid cycle in the visual process**

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Cones photoreceptors are responsible for color vision and are commonly used by humans for normal daily function. In retinal degenerative diseases, rod photoreceptors typically deteriorate more rapidly than cone photoreceptors. However, in the *Rpe65^{-/-}* mouse, a model for Leber's congenital amaurosis, cones degenerate much more rapidly than rods. In this model, the retinoid processing pathway is disrupted and 11-*cis* retinal is not generated. The rod opsin is present and function can be restored in mice as old as 18 months. However, the cone opsins are not detectable at the protein level after 8 weeks. Other cone specific genes are also downregulated.

To follow cone functionality, the *Rpe65^{-/-}::Rho^{-/-}* mouse, in which rod opsin is eliminated, was used. P10 mice were injected intraperitoneally with 11-*cis* retinal and maintained in darkness. At P25, cone function was assessed using ERGs; cone opsin survival was determined immunohistochemically; and cone opsin levels were obtained by quantitative RT-PCR. In untreated P25 animals, cone density and transcript levels of cone opsins were drastically reduced, but a minute cone ERG was detected, suggesting that the remaining cones are functional. Confocal microscopy showed that the cone opsins are mislocalized, suggesting that their transport to the outer segments is impaired. Administration of 11-*cis* retinal increased transport of cone opsins to the outer segments and preserved cones anatomically and functionally. We suggest that the ligand is required during cone opsin synthesis for successful trafficking, and that without 11-*cis* retinal, cones may degenerate due to opsin mislocalization. These results may have important consequences for treating cone dystrophies.

IL58**Visual pigment coexpression. A means of color cone development and regeneration?**

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In contrast to trichromatic primates, most mammals possess two populations of cones (dichromacy with short- and middle wavelength sensitive visual pigments). Recent investigations show that in a number of species another cone population exists that coexpresses both S- and the M-opsins (dual cones). The occurrence of dual cones in the developing retina has been reported on by our group, representing a transitory state in maturation of M-cones, yet the function of dual cones remained unknown in adults.

The house mouse (*Mus musculus*) is the best known species in which dual cones occur even after photoreceptor differentiation has been stopped. In this species M- and S-cones occupy opposite retinal halves and dual cones occur mainly in the transitional zone between them. In the rabbit, guinea pig and vole similar retinal cone distribution has been reported with dual cones restricted to a horizontal zone at the junction of two retinal fields. Interestingly, the Siberian hamster and the pouched mouse exhibit a uniform cone population made up exclusively of coexpressing cones, consequently no signs of retinal division is present.

In another rodent (*Otomys unisulcatus*), coexpressing cones that morphologically resemble those of the developing eye occupy the dorsal peripheral retina. Can it be that some continuously differentiating stem cells survive in this region, and the potential of retinal maturation is maintained uninterrupted through adulthood? To test this hypothesis, immunocytochemistry with Proliferating Cell Nuclear Antigen was performed. PCNA is a well known marker of cell division, with strong immunolabeling of cells in the S-phase of mitotic cycle.

The reported PCNA-positivity underlines that stem cells might indeed function continuously in the peripheral retina, and the developing photoreceptors integrate into the retinal mosaic. The existence of mitotic cells highlights the possible connection

between developmental events and double stained elements in adults.

PL59**Signal transduction in keratinocytes under UV-A radiation**

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Ultraviolet A radiation (UVA) exerts detrimental effects on human skin. It is thus important to study the molecular mechanisms underlying these effects. In previous years we have shown that exposure of primary human epidermal keratinocytes (NHK) to UVA at physiological doses leads to increased gene expression such as ICAM-1, which is mediated through activation of transcription factor AP-2 (PNAS 1996, 93:14586-14591). Subsequently we have identified a non-enzymatic triggering of the ceramide signaling cascade as the initiating step in UVA radiation-induced signaling (EMBO J 2000, 19:5793-5800). These signaling ceramides act on mitochondria and induce the release of non-apoptogenic amounts of cytochrome C, which through a redox regulation causes activation of AP-2 (J Biol Chem 2003, 278:47498-47507). Next, we have shown that the UVA-induced generation of ceramide occurs at the level of the cell membrane sphingomyelin through a process that involves the generation of singlet oxygen. Within the plasma membrane, sphingomyelin is not equally distributed, but preferentially present in microdomains, also called "rafts", which additionally contain high amounts of cholesterol. Our studies indicate that UVA-induced signaling involves cell membrane lipid rafts. This assumption is based on the observation that (i) alteration of the cholesterol content of cell membrane lipid rafts dramatically affects the capacity of NHK to mount a UVA response and (ii) that gene knock-down of the raft-associated protein caveolin-1 by means of siRNA almost completely inhibits UVA radiation-induced gene expression. We have therefore started to analyze the mechanisms through which UVA activates Caveolin-1. Our most recent results indicate a critical role for src kinase yes and fin in this context. The identification of cell membrane lipid rafts and raft-associated proteins as integral components of the UVA-induced stress response provide the basis for the development of novel strategies to protect human skin against UVA-induced detrimental effects.

IL60**Photosensitization in a confined membranal phase: uptake, topography and photochemistry of sensitizers in membranes**

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Most sensitizers that are used for Photodynamic Therapy (PDT) are hydrophobic or amphiphilic and are taken-up by cellular lipid membranes. This passive or active uptake depends, among others, on the physical state of the membrane, namely its composition, temperature, phase, fluidity, electric potential etc. Membrane-localized damage can be caused only while photogenerated is diffusing inside the membrane and can oxidize proteins and lipids. Deeper insertion of the sensitizer in the membrane increases singlet oxygen's dwell time in the membrane and enhances the sensitizing efficiency. We synthesized several modified porphyrin molecules, possessing varying lipophilicities, which are anchored at the lipid:water interface, with the porphyrin core located at different vertical depths in the membrane. A depth-dependent effect on the photosensitization efficiency was observed, even when the depth changed by merely a few Angstroms. Additives that rigidify membranes, such as DMPC or cholesterol, affect the location of the porphyrins in the membrane and thus their efficiency. Strong changes in sensitizing efficiency as a result of changes in temperature, phase transition or fluidity were observed. In addition to the vertical location of the sensitizer in the membrane, we also studied the correlation between lipophilicity parameters, the extent of uptake by lipid membranes and sensitization efficiency in the

membrane. We thus point to the possibility of simple and small modifications to sensitizer molecules that would amplify their efficiency as membrane-bound sensitizers. The manifestation of the topographic modifications of the sensitizers in cellular photokilling will be demonstrated.

IL61

Steady-state and dynamic interactions of photosensitizers with plasmatic proteins and membranes

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The reactive species produced by photosensitization poorly diffuse and their action is confined to the vicinity of the photosensitizer. Thus, subcellular localization of photosensitizers is a key determinant of their biological efficacy. The cellular uptake may involve passive diffusion through the cytoplasmic membrane and/or endocytosis that can be non- or receptor dependent. Association of photosensitizers to low-density lipoproteins (LDL) favors the last route through cellular receptors to LDL. Besides, it is well known from *in vitro* studies that binding to albumin significantly decreases cellular uptake. The aim of the present communication is to identify the parameters governing cellular uptake and subcellular partition of porphyrins and related macrocycles. Three photosensitizers are considered: deuteroporphyrin (DP) a dicarboxylic porphyrin, chlorin e6 with three carboxylic chains, and disulfonated aluminium phthalocyanine (AlPcS2). Only carboxylic compounds can undergo neutralization of their side-chains in a range of pH relevant to physiological values.

Phospholipidic small unilamellar vesicles (SUV) are used as models of membranes. The affinity of photosensitizers for SUV, LDL and albumin are considered as well as the dynamics of the interactions. Fluorescence is used to monitor the binding of the photosensitizer to these various components. Attention is given to dynamic aspects, in particular to the rate of transfer through membranes. Various parameters including the phospholipid bilayer thickness, the presence of cholesterol and the charge of the tetrapyrrolic control these rate constants.

The data obtained on these simple systems can be used to understand subcellular localization of photosensitizers as shown by studies carried out on fibroblasts. Although LDL's are important vectors of photosensitizers, the subcellular localization appears to be directly related to the dynamics of transfer of photosensitizers through membranes.

IL62

Distribution properties of hematoporphyrin in the mitochondrial permeability transition pore

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Mitochondria play a key role in initiating the cell death. A major player in this process is the mitochondrial permeability transition (PT) pore, a proteinaceous channel, permeant to any molecule of ≤ 1.5 kDa, that opens in the inner mitochondrial membrane, most notably as a result of calcium overload and oxidative stress. A current model suggests that PT pores form at contact sites between inner (IMM) and outer (OMM) membranes by association of the matrix cyclophilin D (CypD), the IMM adenine nucleotide translocase (ANT), the OMM peripheral benzodiazepine receptor (PBR) and voltage-dependent anion channel (VDAC), and possibly other proteins. Dicarboxylic porphyrins, including hematoporphyrin (HP), have been claimed to represent the predominant high-affinity endogenous ligands for PBR and, through this protein, to interact also with VDAC and ANT. Thus, it

is plausible to suppose that these porphyrins accomplish a strategic localization in the pore complex. This hypothesis is supported by the following phenomena concerning HP microenvironment, which are correlated with the pore activity: (i) singlet oxygen photogenerated in HP-loaded mitochondria prevents the opening of the MPT pore. The targets for photodynamic action are pore-regulating histidines that undergo selective degradation. Irradiated mitochondria, in fact, can still undergo the PT when treated with other pore inducers; (ii) during HP photodynamic action, neither electron transport nor membrane potential are severely impaired whereas oxidative phosphorylation is inhibited. The damage mainly involves ANT; (iii) after addition of the pore inducer, perturbations of HP-binding proteins are observed. Such perturbations precede the opening of the pore and are strongly inhibited by protonation of the critical histidines. We suggest that HP preferentially accommodates in mitochondrial domains of ANT containing pore-regulating histidines which undergo a structural remodelling before the opening of the pore.

IL63

Towards a targeted photodynamic therapy?

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Photodynamic therapy (PDT) is now well established as a clinical treatment modality for both neoplastic and non neoplastic diseases. However, the drugs used still display a poor selectivity towards the intended targets. Several strategies have been proposed to improve the selectivity of sensitizers. Among them, structure modifications induced by glycoconjugation tetrapyrrolic macrocycles appears as a viable means to achieve a targeting of the sensitizer towards tumor cells. Besides modification of the lipophilic/hydrophilic balance, glycoconjugation provides the possibility for specific interactions with sugar receptors -so called lectins- that have been shown to be overexpressed by certain malignant cells. Efforts have been focused in our laboratories on the preparation of new tetrapyrrolic macrocycles bearing a cellular recognition element linked to the macrocycle. A series of neutral glycoconjugated tetrapyrrolic macrocycles, polyamino, peptide and pseudopeptide analogous are undergoing *in vitro* evaluation. The effect of glycoconjugation on internalization processes, cellular sublocalization, cellular degradation and photodynamic efficacy will be examined. The involvement of a specific affinity towards glycoside receptors will be emphasized and an original method for the determination of the nature of glycoside membrane receptors based on the use of magnetic nanoparticles coated with glycoalbumine will be presented. The case of porphyrins bearing α -mannosyl or β -galactosyl moieties via a diethylene glycol spacer will be particularly developed with regard to a possible use of PDT in the treatment of retinoblastoma.

OC64

Enhanced selectivity of tri-component pro-drugs: initial *in vitro* and *in vivo* results

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We have developed a novel enzyme-activated drug delivery approach in order to enhance the therapeutic index of anti-tumour drugs, either chemo or photochemotherapeutic agents (US Patent No. 5,618,790). The approach involves the use of a soluble tri-component pro-drug that contains an active agent, a solubilizer, and a linker. The linker is chosen so as to be cleaved at a specific site by over-expressed proteolytic enzymes that predominate at the tumour invading front. Such enzymes are responsible for breaking

down the protein cellular matrix in order to permit the tumour to advance. The first tri-component pro-drug tested included mesoporphyrin IX as the active agent and tetralysine as a solubilizer. These two elements were linked via a polypeptide sequence Gly-Pro-Leu-Gly-Pro-Ala that can be recognized by specific proteolytic enzymes such as collagenase (1A or IV). Upon such cleavage, the solubility of the mesoporphyrin IX decreased rapidly, which could be monitored via its decreased fluorescence intensity. Under similar experimental conditions, other proteases not specific to this polypeptide sequence, such as papain and chymotrypsin, yielded only slow or negligible loss of fluorescence. Full fluorescence intensity of solutions could be restored by addition of 8M urea, indicating that the enzymes had not destroyed the mesoporphyrin. Fresh human blood did not cleave the collagenase-sensitive linkage. *In vivo* activity of such enzyme activity was measured in mice that had been given intra-dermal injections of *C. perfringens* (which secretes large quantities of collagenase). When the pro-drug was administered, a significant change in fluorescence intensity was observed at the site of *C. perfringens* injection. These results demonstrate the principle of selectively delivering anti-tumour drugs to the protease rich tumour site, thereby enhancing drug delivery selectivity.

OC65

Proteolytically-induced photosensitization, a new tool in photodynamic therapy

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For quite sometime, the tailoring of drugs to a specific disease has been only wishful thinking in the field of photodynamic therapy (PDT), and except for the use of 5-aminolevulinic acid and derivatives, prodrug design has been absent. However, our recent discovery of a new class of photosensitizers makes this approach now feasible. In this context, proteolytically-induced photosensitization is a process in which enzymatic activity is used to activate a photosensitizer (PS). We have studied this phenomenon and determined its feasibility as a new therapeutic tool. For instance, by targeting pathologically associated enzymes, we have developed polymeric-photosensitizer conjugates with exquisitely selective anticancer potential. The idea is based on the use of polymeric carriers loaded with varying amounts of pheophorbide a, as PS, via cleavable peptidic linkers. In the absence of appropriate enzymes, these conjugates lack photosensitizing activity due to efficient energy transfer among the PS moieties attached to the polymeric backbone. However, upon selective enzymatic degradation within the pathological environment, the PS units are cleaved from the conjugate and their photoactivity restored. Under these conditions, the related PS fluoresces and generates reactive oxygen species (ROS) when irradiated with light ablating abnormal tissue. We investigated the photophysical and photochemical properties of these photosensitizer conjugates with respect to PS loading and also explored the use of tethered PEG side chains to improve on their physicochemical characteristics. Thus, proteolytically-induced photosensitizing agents can be made to specifically fit with the requirements of a given therapeutic application and represent a breakthrough in photochemotherapy.

OC66

Effects of photosensitizers nanoencapsulation and of the size of the nanoparticles on photothrombic activity for the treatment of choroidal neovascularisation by photodynamic therapy

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Photodynamic therapy (PDT) is the most promising therapy used for the treatment of choroidal neovascularization due to age-related

macular degeneration. To be useful as a therapy, some selectivity between the target tissue and the neighbouring healthy structures must be achieved. This could be obtained by choosing a suitable delivery system for the photosensitizer (PS). Hence, the main goal of the present study was to develop a polymeric carrier system such as nanoparticles (NP) for PS intended to be intravenously administered, capable of increasing the therapeutic index of the PS and devoid of adverse effects. To assess the effect of the size of NP in terms of photothrombic activity, different sizes of TCPP-loaded NP (120-350 nm) were synthesized using the salting-out technique with biodegradable polymer. For PDT studies, chorioallantoic membrane (CAM) of fertilized chicken eggs were placed under an epi-fluorescence microscope and an autofluorescence image of surface was recorded. Subsequently, injections of the different batches of TCPP-loaded NP (0.5 mg/kg body weight (b.w.)) were performed in situ under the microscope and their circulation were observed by fluorescence microscopy. Following the homogenous distribution of the photosensitizer in the blood circulation, PDT was performed using a filtered Hg-arc lamp at 420 ± 20 nm. Light dose of 10 J/cm^2 was applied to the CAM. Then, the eggs were returned to the incubator for 24 hours and vascular occlusion was observed by means of fluorescence angiography, performed by injection of sulforhodamine 101. Fluorescence angiographies pre- and post-PDT were compared and scored according to an arbitrary score. The PDT-induced vascular damage proportionally decreases with the size of the TCPP-loaded NP. Depending on the size, the NP could be transported and accumulated into endothelial cells via different mechanisms and/or rate leading to different intracellular localization leading to different vascular damages.

OC67

Vectorisation of photosensitisers (protoporphyrin IX and hypericin) by vesicles expelled by *Dictyostelium discoideum* cells: a new vectorisation tool for cancer diagnosis and photodynamic therapy (PDT)?

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During unicellular growth and aggregation, the eukaryotic microorganism *Dictyostelium discoideum* expels vesicles. First recognised as detoxification agents, these vesicles behave also as intercellular messengers. The present work focuses on the use of these biological vesicles to carry two photosensitisers, protoporphyrin IX and hypericin. When grown in suspension in the presence of 5-aminolevulinic acid, *Dictyostelium* cells biosynthesise protoporphyrin IX and expel vesicles loaded with the photosensitiser. When grown, either in suspension or as adherent cells, in the presence of hypericin, *Dictyostelium* cells release in the extracellular medium vesicles loaded with the non-metabolised photosensitiser. A first characterisation of the vesicles prepared by differential centrifugation was carried out by SDS-PAGE and electron microscopy. The amount of hypericin loaded in vesicles, originating from adherent cells, was studied by spectrofluorimetry. In the presence of $25 \mu\text{M}$ hypericin, the cell growth, in the dark, remained unaffected up to 72 h. At different times of incubation, cells and the corresponding extracellular vesicles were studied by flow cytometry and microspectrofluorimetry, respectively. The photosensitiser was shown to be quickly cell internalised and progressively released via the expelled vesicles. With the aim of using *Dictyostelium* vesicles as a vectorisation tool for early cancer diagnosis and photodynamic therapy, *in vitro* and *in vivo* experiments are currently under study.

OC68**Photodynamic activity of an estrogen-pheophorbide (a) conjugate in human breast cancer cells (MCF7)**

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Photodynamic therapy (PDT) is a new and evolving modality for the treatment of cancer which involves the selective uptake and retention of a photosensitizer in tumor cells. The activation of sensitizer by light leads to tumor necrosis. Because photofrin II, currently used in PDT is a complex mixture of monomers, dimers and oligomers, it shows some problems of reproductibility. These problems have justified the need for new drugs development devoted to find new pure and more selective photosensitizers which absorb at long-wavelength. In this context, a porphyrin, the pheophorbide (a), was conjugated to estradiol via alkoxy spacers of various length.

An *in vitro* biological study performed on MCF7 cell line, shows a specific nuclear localisation of the photosensitizer due to the presence of the estradiol residue. Moreover, these new compounds display a phototoxic activity eight time higher than that of the unmodified pheophorbide. This targeting strategy appears to be promising for the design of new photosensitizers for the photodynamic therapy.

IL69**Anti-oxidants and natural UV filters in skin protection**

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None of the current sunscreens are perfect and the search continues for improved formulations. We report two novel approaches to this problem. The first is based on substances extracted from lichens in areas of Chile where there is ozone depletion and the second on carotenoids-anti-oxidant systems that are efficient quenchers of free radicals and singlet oxygen. We aim to obtain both an immediate and effective skin protection against sunburn and protection of skin connective tissues, thus reducing long-term damage. These will contribute to protection against skin cancer.

Combinations of anti-oxidants (e.g. lycopene, β -carotene, vitamins E and C) are shown to give a synergistic benefit in the protection skin fibroblasts against UV and against the damage due to oxy-radicals (e.g. NO_2^{\bullet} and singlet oxygen). Protection factors varied from 5 – 17 with the most efficient being based on lycopene.

Results on the lichen compounds, e.g. usnic acid and calycin show that they do not generate singlet oxygen (essential for a sunscreen) and that they quench free radicals. We showed vitamin C could repair lichen sunscreen radicals (generated quenching radicals). Reactions with vitamin C were more efficient for usnic acid than calycin. In biological experiments we obtained protection factors for cellular membranes of 4.5 for usnic acid (against UVB) and 3.2 for calycin (against UVA).

Future studies will show if lichen extracts are efficient singlet oxygen quenchers and will extend the range of free radicals studies. I thank Drs R. Edge, S. Navaratnam, F. Rancan and Professors E Fernández and G Truscott for collaboration. Experiments were carried out at the FRRF of the CLRC Daresbury Laboratory, Warrington, UK, supported by EC through the "Improving Human Potential" Transnational Access to major Research Infrastructures (Contract HPRI-CT-2002-00183).

IL70**What's new in sunscreens**

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Sunscreens are well documented to prevent acute effects of excessive sun exposure. The sun protection factor is directly related to the ability of a sunscreen to prevent ultraviolet-induced erythema. Appreciation of the detrimental effects of ultraviolet A

(UVA) has led to the development of products able to protect against such wavelengths. Greater understanding of the ability of UVB and UVA to cause local and systemic immunosuppression has led to broad spectrum sunscreens enabling such effects to be blocked. The addition of active agents to sunscreens to actively prevent photosensitivity such as polymorphic light eruption has heralded a new way of perceiving sunscreens as active therapeutic products and not just preventing access of UV to the skin. Many new active agents are emerging some showing *in vivo* efficacy others still being tested *in vitro*.

IL71**What's new in the photodermatoses**

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The photodermatoses comprise the probably immunologically mediated polymorphic light eruption (PLE), actinic prurigo (AP), hydroa vacciniforme, chronic actinic dermatitis (CAD) and solar urticaria, the DNA repair-defective disorders, particularly xeroderma pigmentosum, chemical- and drug-induced photosensitivity and the light-exacerbated dermatoses. PLE, a common, papular, itchy eruption of some or all exposed areas of usually young women within hours of sun exposure, lasting days, is very likely a delayed-type hypersensitivity (DTH) reaction against UVR-induced endogenous cutaneous antigen, and genetically based on a low penetrance susceptibility allele carried by up to 70% of people. This leads to reduced normal UVR-induced cutaneous immunosuppression during DTH induction, namely the first PLE attack, but not thereafter during elicitation, namely subsequent attacks. AP, a persistent, excoriated, sometimes eczematized, eruption of mostly exposed sites in children appears to be genetically prolonged PLE, demonstrating the probable modifying factor, HLA tissue type DR4 or subtype DRB1*0407 in most patients. CAD, a persistent eczematous, sometimes pseudolymphomatous, eruption of exposed areas of usually older men, sometimes younger atopsics, appears to be an allergic contact dermatitis-like reaction against presumably endogenous cutaneous photoantigen, conceivably sometimes DNA or a related molecule. A new treatment sometimes effective is topical tacrolimus. Xeroderma pigmentosum, a DNA repair disorder with early photoageing and photocarcinogenesis, seems considerably improved by a liposomally delivered, bacteriophage-derived topical DNA repair enzyme, T4 endonuclease. Finally, light-exacerbated seborrhoeic eczema appears very common, resembling PLE by history but demonstrating seborrhoeic eczematous features, and seeming to respond well, even when severe, to assiduous therapy of the underlying condition.

IL72**Photodynamic therapy in dermatology: recent advances**

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Topical photodynamic therapy is now well established for the treatment of various oncologic and non oncologic skin diseases. The development of well tolerated cutaneous photosensitizers like 5-aminolevulinic acid or its methyl ester has enhanced the interest for this treatment modality in dermatology. Red light emitted by incoherent lamps or diodes is now widely available by physicians. Actinic keratosis, superficial basal cell carcinomas and Bowen's disease are recognized indications in the field of dermato-oncology. Recent publications of comparative studies have reported equivalent cure rates to standard and reference treatments for superficial lesions. Moreover a therapeutical benefit of PDT has been reported in a wide range of inflammatory dermatosis such as acne vulgaris, granuloma annulare, localized scleroderma or benign familial pemphigus. Very low rates of side effects are reported by patients whereas cosmetic outcome is generally considered as very good or excellent. Topical photodynamic therapy should become a

widely accepted treatment in the near future among various fields of dermatology.

OC73

Photodynamic action of red light for treatment of erythrasma.

Preliminary results

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Background: Erythrasma is a superficial cutaneous infection caused by *Corynebacterium minutissimum* and is characterized by fluorescence under Wood's light (UV) due to the presence of porphyrins. These molecules are photosensitizing and we propose to assess efficiency of red light that activates porphyrins (photodynamic reaction) in treatment of this pathology.

Objectives: assessment of effects of photodynamic action of red light in treatment of erythrasma without exogenous photosensitizing molecules.

Methods: eight patients with erythrasma were treated by one illumination (80 J/cm²) by red light (broad band 635 nm) without exogenous photosensitizing molecules. Disappearance or size reduction of lesions were observed two weeks later. If lesions were still present, a second irradiation was proposed with the same method.

Results: preliminary results are presented. Red light irradiation has allowed improvement of lesions: intertrigo complete disappearance was achieved with only one illumination for 2 patients and with 2 illuminations for a third patient. Moreover, in others cases, lesion size reduction was achieved. Eight lesions were precisely assessed with tracing before and after one illumination: there was a size reduction in 7 cases with reduction average of 43% (from 20% to 93%). Treatment tolerance was good.

Conclusion: we report first cases of photodynamic treatment of erythrasma. This technique seems to be an interesting alternative, cheap and easy, to treat this localized infection that uses spontaneous presence of porphyrins in lesions. But the optimal method is still to be defined to improve efficiency.

OC74

Porphyrin photosensitizers, suitable for the photodynamic treatment of tinea infections caused by *Trichophyton rubrum*?

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Background: Dermatophytes are fungi that can cause infections of the skin, hair and nails because of their ability to feed on keratin. Superficial mycoses (tinea infections) are probably the most prevalent of infectious diseases in all parts of the world. One of the most important restrictions of the current therapeutic options is the return of the infection and the duration of the treatment. Recently we demonstrated, using porphyrin photosensitizers, that *in-vitro* a single photodynamic treatment (PDT) was sufficient to achieve a 100% fungicidal effect, viz. killing of the dermatophyte *Trichophyton rubrum*.

Purpose: to evaluate the photodynamic activity of the porphyrin photosensitizers in a situation that mimics the clinical situation of tinea infections we developed an *ex-vivo* model using human stratum corneum (SC). The *ex-vivo* model offers the possibility to apply PDT at different time points during the germination process of *T. rubrum* microconidia and their development thereafter. The porphyrin photosensitizers under study were also evaluated for their mutagenetic potentials and submitted to skin penetration studies.

Results and conclusion: the porphyrin photosensitizers that were proven to exhibit a fungicidal effect *in-vitro* were tested in the *ex-vivo* model. It was demonstrated in this model that the susceptibility of *T. rubrum* to PDT was determined by the time of

application in relation to the developing germinating microconidia. Using the Somatic Mutation And Recombination Test (SMART) for the mutagenicity experiments in absence and presence of broadband white light, it was proven that the porphyrins under study displayed an indication for mutagenicity. Since tinea infections are in general superficial infections they will be extremely suitable for PDT and porphyrins can be considered as suitable photosensitizers if a proper formulation prevents skins penetration.

OC75

Psoralen metabolism and psoralen ultraviolet-A (PUVA) sensitivity: a role for cytochrome P450 CYP1B1

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Exposure of human skin to ultraviolet radiation (UVR) and psoralen UVA (PUVA) generates reactive oxygen species (ROS). Drug metabolising enzymes (DMEs), including cytochrome P450s (P450s) are induced as an adaptive response to oxidative challenge. We have shown that P450 CYP1B1 shows marked individuality in cutaneous expression and inducibility by UVR and PUVA (Smith *et al*, J Invest Dermatol 2003; 121: 390-8). Differences in cutaneous DME expression may therefore influence individuality in response to PUVA. Although P450s are known to metabolise psoralens, it is not known which P450 isozymes are involved and whether variability in cutaneous P450 expression is a determinant of PUVA sensitivity.

We used *E. Coli* co-expressing human CYP1B1 and its redox partner cytochrome P450 reductase (CPR) to investigate 8-methoxypsoralen (8-MOP) metabolism *in vitro*, and used CHO cell lines stably over-expressing CYP1B1 and CPR to investigate whether CYP1B1 expression influences PUVA sensitivity. We demonstrated that CYP1B1 metabolised 8-MOP (turnover rate 0.236 min⁻¹). In addition, CYP1B1-dependent ethoxresorufin demethylase activity was inhibited by 8-MOP (estimated IC₅₀ 21 ± 2 mM). We observed that 8-MOP (0-1000 ng/ml) or UVA (1.5, 4, and 7.5 J/cm²) alone were minimally cytotoxic to CHO cells, although PUVA was cytotoxic. The most marked PUVA cytotoxicity was observed when a UVA dose of 4 or 7.5 J/cm² was used. Using a UVA dose of 4 J/cm² and 8-MOP (0-1000 ng/ml), we demonstrated that the CHO CYP1B1/CPR over-expressing cell line was more susceptible to PUVA than either the parental CHO cell line or the CHO cell lines over-expressing CYP1B1 or CPR alone. These data suggest that CYP1B1 may be an important determinant of 8-MOP metabolism and disposition, and may be involved in cellular responses to PUVA.

We are grateful to the Medical Research Council for funding this study.

OC76

Differential impact of XPD gene mutations associated with either the photosensitive cancer-free trichothiodystrophy or the cancer-prone xeroderma pigmentosum syndrome

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Genetic alteration of the repair of UV-induced DNA lesions (cyclobutane pyrimidine dimers, CPD, and 6-4 photoproducts, 6-4 PP) by nucleotide excision (NER) may result in photo genodermatoses, such as the cancer-prone xeroderma pigmentosum (XP) syndrome or, the cancer-free trichothiodystrophy (TTD) syndrome. We aimed at comparing the impact of mutations in the XPD gene resulting in either the XP-D (R683W) or the TTD/XP-D (R112H) syndrome. We cultured primary fibroblasts and keratinocytes from small skin biopsies of XP-D and of TTD/XP-D

patients. Comparative study of DNA repair kinetics demonstrated faster and better DNA repair capacity of TTD/XP-D cells compared to XP-D cells and hence, a shorter P53 stabilisation in the former. In order to reproduce the 3D cutaneous architecture, we elaborated organotypic TTD/XP-D and XP-D skin cultures. Response to UVB (290-320 nm) irradiation in term of DNA repair and apoptosis, was also better in TTD/XP-D XP-D than in XP-D organotypic skins. We also assessed expression of markers of epidermal homeostasis and revealed alteration or extinction of some epidermal differentiation markers such as filaggrin in XP-D, but not in TTD/XP-D organotypic skin cultures.

Altogether these results indicated that the R112H XPD mutation resulting in the cancer-free TTD/XP-D syndrome are associated to a better vital prognostic of cutaneous cells compared to the XPD R683W mutation characteristic of XP-D cancer-prone syndrome.

OC77

Mitochondrial damage and G1 arrest are the primary events for PUVA-induced apoptosis in human keratinocytes

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Psoralen plus UVA (PUVA) is a highly effective therapy for the treatment of psoriasis and other skin disease. Previous studies have demonstrated that PUVA induces apoptosis in human lymphocytes and in other cell lines, but the precise mechanism(s) are not fully elucidated in detail. In this study we have investigated the biological activity of four psoralen derivatives: 5-methoxypsoralen (5-MOP), 8-methoxypsoralen (8-MOP) Angelicine (ANG) and 4,6,4'-trimethylangelicine (TMA) in immortalized keratinocytes cell line NCTC 2544. The well known antiproliferative effect of psoralens was demonstrated in a wide range of UVA doses and drug concentration. The mechanism of cell death was investigated by flow cytometry. The results show that in keratinocytes the four derivatives after irradiation induce, a block of the cell cycle in G1 phase followed induction of apoptosis as shown by the appearance of a sub-G1 peak. The G1 arrest is concomitant with the increased expression of p21^{Waf/Cip} a protein that induce growth arrest by blocking cyclin-dependent kinases (CDK). These observations prompted us to investigate further the potential activation of the apoptotic machinery after irradiation in the presence of psoralen. In addition psoralen specifically localizes in mitochondria as demonstrated by fluorescence microscopy and after irradiation, mitochondrial dysfunction such as decrease of the mitochondrial transmembrane potential cytochrome c release and Reactive Oxygen Species (ROS) production caused by the opening of mitochondrial permeability transition pore occurs. Thus, these results suggest that a mitochondrial damage upon PUVA treatment may occur and this event leads to an apoptotic cell death. The role of the caspases in terms of apoptosis induction has also been studied and the obtained result show for all the four derivatives an activation of caspase-9 and 3 but not caspase 8.

IL78

Development of fluorescence cystoscopy with Hexvix®: the road from *in vitro* studies to clinical results

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The prognosis of superficial bladder cancer is linked to tumor multiplicity and the presence of occult flat tumors such as high grade dysplasia and carcinoma in situ. Therefore, a sensitive and specific detection of these lesions, which are quite invisible in

white light cystoscopy, is of high importance. Early attempts to solve this medical problem were based on the use of aminolevulinic acid (ALA) induced protoporphyrin IX (PPIX), a precursor with limited performances. Our research in this context aimed at optimising the PPIX accumulation within cancerous tissues. We focused our attention on the study of various ALA esters administered at different concentrations. Since these studies could not be performed in the clinic, micro dissected specimens of porcine urothelium were mounted into a thermostabilized (37°C), bicameral transparent culture box and placed under a modified fluorescence microscope. The analysis of the PPIX accumulation kinetics and distribution across the urothelium enabled to define the optimal type of ALA derivative and administration conditions.

Our first clinical investigations indicated that with hexylaminolevulinic acid (HAL) a significant (> 2x) increase of PPIX fluorescence intensity could be observed using 20-fold lower concentrations (4 - 8 mM) as compared to ALA. In addition, a study on 143 patients comparing two protocols, with different HAL instillation times, confirmed that the reduction of this time to less than one hour does not reduce the performance of the HAL fluorescence cystoscopy in terms of sensitivity and specificity. This represents a real advantage in daily clinical practice since the use of ALA requires a much longer instillation time.

The combined efforts of *in vitro*, preclinical and clinical studies in Lausanne, in which the HAL was selected, eventually led to the phase II and III clinical trials undertaken together with Photocure ASA, and to the approval of HAL in 27 European countries in March 2005.

IL79

Time domain optical mammography and spectroscopy of the breast

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The absorption and scattering properties of breast were assessed *in vivo* over a broad spectral range (600-1100 nm), with a unique instrumentation for the non-invasive optical characterization of highly diffusive media by means of time-resolved transmittance and reflectance spectroscopy. The absorption properties provided information on tissue composition and the scattering properties on its structure. We investigated the correlation between optical properties and tissue composition, the spatial heterogeneity of tissue, the dependence of the optical properties on physiological changes.

The information derived from spectroscopy was applied to the development of the first optical mammograph operating in the time domain at 4 to 7 wavelengths between 635 and 975 nm (red to near infrared). Absorption and scattering images are collected in compressed breast geometry, deriving information on tissue content of oxy- and deoxyhemoglobin, water, and lipids, and on tissue density.

The optical mammograph was tested in a clinical trial on 200 patients with malignant and benign lesions, within the EU project "Optimamm". Optical features that characterize the different breast structures (e.g. mammary gland, blood vessels, adipose regions) and lesion types were identified. Typically, cancers are recognized by the strong absorption in the red, due to the neo-vascularization, while cysts are detected based on the low scattering value, related with their liquid nature. The optical appearance of fibroadenomas is more mixed. Detection rates for cancers and cysts in one view are 96% and 90%, respectively, while they decrease to 80% and 83%, if detection in two views is required. Correlation between demographic parameters (e.g. age, body mass index) and tissue composition and structure, as derived from optical data, was also investigated.

IL80**Studying brain physiology and pathology with non-invasive near infrared spectroscopy**

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By means of non-invasive near-infrared spectroscopy (NIRS) the cerebral concentration changes in oxygenated and deoxygenated haemoglobin can be determined. NIRS has become popular to study the physiological basis of functional imaging techniques, such as functional magnetic resonance imaging (fMRI). Currently, further neuroimaging applications have been assessed with subjects and stimuli which are not suitable for MRI.

Despite the success of these research applications a wide-spread diagnostic with NIRS is hampered due to a poor specificity caused by i) the difficulty of an absolute quantification, ii) confounds by 'systemic', extracerebral oxygenation- and blood-volume-changes and iii) a cross talk between the two haemoglobins.

This talk will focus on ours and other groups most recent technological advancements to overcome these issues: i) Topographic (multi-channel) imaging allows to reduce systemic effects. ii) 'Depth-resolution' improves quantification and thus allows to reduce extracerebral confounds. iii) The transcranial detection of fluorescence opens a new door for molecular imaging and thus potentially increases the patho-physiological specificity of NIRS.

IL81**High speed optical coherence tomography in ophthalmology**

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Optical Coherence Tomography (OCT) uses infrared light interferometry to obtain sections of tissues which weakly absorb and scatter infrared radiation. Because of the high axial (from 1 to 15 micrometers) and lateral (about 20 micrometers) resolution, and the relatively high transparency of ocular tissues for near infrared radiation, as well as the non-contact and non-invasive way the measurement is performed, the OCT technique is well suited to studying the microstructure of the eye.

The traditional variant of OCT – the time domain version- has already been implemented in instruments which are now commercially available. Our, recently developed Fourier domain variation of OCT enables an almost 100 fold improvement in imaging speed over the traditional method. The short acquisition time as well as evident reduction of patient discomfort, offers several advantages. There is an increase in the quality of images brought about by the noticeable reduction of image-blurring motion artifacts and the possibility of high density of A-scans. Fast acquisition permits collection of amounts of data sufficient for 3-D imaging in a time acceptable for the patient as well as registration of sequential frames for a tomographic movie to reveal dynamics of elements of the microstructure. Fast acquisition and high phase stability also expand the range of flow velocities measured by this technique.

Three dimensional OCT imaging provides a comprehensive characterization of retinal microstructure which promises to better elucidate structural changes associated with retinal disease, and to improve early diagnosis and monitoring of disease progression and response to treatment.

In this report, we present the basic physical background of Optical Coherence Tomography, and its strengths and limitations, as well as recent advances in Fourier domain OCT. To demonstrate the capability of this technique, we present cross-sectional images of the retinas and corneas of normal and pathological human eyes *in vivo*.

OC82**Autofluorescence characterization of human steatotic liver**A.C. Croce¹, U. Bareato², D. Neri², U. Cillo², I. Freitas¹, G. Bottiroli¹¹Histochemistry and Cytometry, IGM-CNR, Department Animal Biology, University, Pavia; ²Ist General Surgery Department, University, Padoa, Italy

The gap between demand and availability of livers for transplantation is continuously increasing leading to accept marginal livers. Organs either from elderly donors, or suffering for prolonged ischemia, or affected by steatosis, although not optimal, may be the only viable alternative for patients in waiting lists. Steatotic livers are characterized by accumulation of lipids, mainly triglycerides, in hepatocytes cytoplasm. The fat percentage/liver weight leads to steatosis classification as mild (<30%), moderate (<60%) and severe (>60%). Since lipids increase liver susceptibility to ischemia-reperfusion injures of preservation procedures, only a mild-to-moderate steatosis degree is acceptable. At present, only biopsy histology provides a reliable diagnosis of steatotic degree, to prevent explantation of unacceptable organs. Autofluorescence properties - already exploited for an *in situ*, real time, monitoring of liver metabolism under experimental conditions - were investigated in human steatotic livers for diagnostic purposes, with particular reference to lipids. Liver samples were collected during surgery, frozen in liquid N₂, and preserved at -80°C. Unfixed, unstained cryostatic sections were submitted to microspectrofluorimetry and imaging analysis (exc.366 nm), or to H&E staining for diagnosis. Typical lipid vacuoles exhibited an intense, red shifted emission, with faster photofading kinetics, with respect to normal parenchima. Comparable autofluorescence properties were found through fiber-optic probe measurements in bulk tissue specimens, that could be related to focal lipid localization in dependence of the steatosis degree. A correlated analysis of signal amplitude, spectral shape and photofading kinetics of autofluorescence emission promises can provide a promising approach to define the degree of lipids accumulation. The development of suitable algorithms calculation would provide the basis to set up real-time techniques for steatosis assessment.

OC83**Influence of cholesterol on cell membrane dynamics probed by fluorescence spectroscopy and imaging**P. Weber¹, H. Schneckenburger^{1,2}, M. Wagner¹, W.S.L. Strauss²¹Hochschule Aalen, Institut für Angewandte Forschung, 73430 Aalen, Germany; ²Institut für Lasertechnologien in der Medizin und Messtechnik an der Universität Ulm, Helmholzstr. 12, 89081 Ulm Germany

Membrane dynamics –including membrane stiffness and fluidity- are important features of living cells. These parameters seem to be important due to their large impact on cellular uptake and release of various metabolites or pharmaceutical agents. They are measured by a combination of conventional and total internal reflection fluorescence microscopy (TIRFM) as well as fluorescence decay kinetics. The generalized polarization (GP, characterizing a spectral shift which depends on the phase of membrane lipids), the time constant of fluorescence anisotropy (τ_r) as well as the fluorescence lifetime (τ) of the membrane marker laurdan revealed to be appropriate measures for membrane stiffness and fluidity. GP generally decreased with increasing temperatures and was always higher for the plasma membrane than for intracellular membranes. The impact of cholesterol content on membrane stiffness and fluidity was now examined using laurdan and defined protocols of cholesterol depletion and enrichment. In addition to the decrease of GP with increasing temperature, the GP values showed a pronounced increase upon enrichment and a decrease upon depletion of cholesterol. Cholesterol content had also a large impact on fluorescence lifetime and fluorescence anisotropy measured in the subnanosecond range. GP, τ and τ_r were determined as integral values of single cells or small cell collectives and were also

displayed as images using an image intensifying camera with subnanosecond time resolution.

OC84

Comprehensive study of the phenomenological mechanisms involved in autofluorescence bronchoscopy

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Autofluorescence bronchoscopy is a useful tool for early cancer detection. The sensitivity of this approach has been shown to exceed the sensitivity of conventional white light bronchoscopy by about a factor of two. However the phenomenological mechanisms involved in this diagnostic procedure are poorly understood. In order to elucidate these mechanisms, we conducted a series of temporally and spatially resolved fluorescence spectroscopic studies during endoscopy.

These fluorescence lifetime and spatially resolved measurements were performed with a "self-built" time-resolved optical fiber-based spectrofluorometer and with an endoscopic imaging system (Diagnostic AutoFluorescence Endoscopy "DAFE") developed in collaboration with the company Richard Wolf GmbH, Knittlingen, Germany, respectively. Biopsies were taken after the measurements and the tissue pathology was confirmed by a histopathologist in all cases. In order to assess the relative value of the tissue autofluorescence yield with the DAFE, we designed an endoscopic reference fluorescence phantom with tissue-like fluorescing and backscattering properties. The probe was inserted in the tracheo-bronchial tree through the biopsy channel of a bronchofiberscope. Images of the probe placed next to the tissue were recorded and analyzed offline.

Fluorescence lifetime measurements in the patients' tracheo-bronchial tree showed no difference when conducted on normal or abnormal tissue sites. Even if the abnormal tissue site showed a chromatic autofluorescence contrast with the DAFE, no difference in fluorescence lifetimes was observed.

These results indicate that the mechanisms involved in the autofluorescence detection of early cancers in the human bronchi are dominated by architectural effects, involving changes of the vascular relative volume in the submucosa. The concentration of fluorescing molecules, quenching and changes in the tissue optical coefficient seem to play only a secondary role, if any.

OC85

Laser induced delayed luminescence from normal fibroblasts and melanoma cells: spectral analysis as a non-invasive diagnostic tool

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With the aim of developing new non-invasive technique able to discriminate between normal and carcinogenic cells in human tissues, spectral analysis of the Delayed Luminescence (DL) emitted by cultured cell suspensions of human fibroblasts and melanoma cells was performed. Previously it was shown that DL, that is the low-level photo-induced emission which lasts longer time after switching off the excitation light, was closely connected to the structure of the emitting systems and in biological system could be connected to the formation of soliton states inside the quasi uni-dimensional polymeric chains that constitute the cytoskeleton. The measurements of the DL from cell cultures were performed by using a dedicated set-up. After ultraviolet-A laser

irradiation (wavelength 337 nm, 5 ns pulse width, 100±5 µJ/pulse), the spectral analysis, due to the very low intensity of DL, was performed by using broad band interference filters at wavelengths 460, 509, 567, 645, 686 nm, respectively. Intensities and kinetics of the spectral components were compared for normal and tumor cells at physiological temperature. The relative intensities of the various spectral components exhibited significant differences for fibroblasts and melanoma cells with high reliability. In particular the ratio between the 645 nm and the 460 nm DL components was 3.7 greater in tumor than in normal cells. Significant differences were also present in the decay curves $I(t)$ and were emphasized by evaluating, starting from the experimental values of the intensities I_i at time t_i , the dimensionless decay probability curves $P(t)$. It appeared that the $P(t)$ trends were quite different for fibroblasts and melanoma cells, especially for the long wavelength components which, in contrast to fibroblasts, in melanoma cells exhibited a marked maximum in the shorter time region. The obtained results suggest to use DL as a parameter for cell identification.

IL86

Interactive effects of UVR and mixing in aquatic environments

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The irradiance received by phytoplankton in the water column is conditioned by vertical mixing, and thus the depth of the upper mixed layer (zUML) plays an important role at the time to assess the effects of solar radiation on these organisms. With shallow UMLs, the mean irradiance received by cells is higher than that found in deep UMLs, providing that other variables are about the same. Earlier studies have found variable effects of mixing on primary production, so that vertical mixing enhanced, decreased or even had no effects; however, these experimentation addressed only the effects of PAR (400-700 nm).

Experiments conducted in the Southern Ocean determined that phytoplankton photosynthesis was more inhibited by UVR (280-400 nm) due to mixing than samples exposed to fixed irradiances, with assemblages responding to dose rather than to irradiance. Experiments conducted in mid latitudes in Patagonia and tropical areas of China highlighted the importance of not only zUML but also the speed of vertical mixing.

Shallow UML (0.6 of the euphotic zone, zEu) resulted in significant UVR-induced inhibition but the same assemblage was able to use UVR as source of energy for photosynthesis with deep UML (> 0.9 of zEu). Fast mixing within the same UML, resulted in an enhancement of carbon incorporation in samples exposed to full solar radiation (UVR+PAR) as compared to that exposed to PAR only, suggesting also the use of UVR for photosynthesis. Thus, experiments to evaluate the interactive effects of vertical mixing and UVR should be done to evaluate how much "standard" fixed-depth incubations deviate from *in situ* primary production.

IL87

Photoreceptors in marine diatoms

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Marine diatoms are the most successful group of photosynthetic eukaryotes in the oceans, and contribute close to one quarter of global primary productivity on Earth. As such, they are likely to possess sophisticated systems for optimizing their photosynthetic capacity under changing light conditions. We have characterized the expression of photosynthetic genes from marine diatoms and have found evidence for perceptory mechanisms for blue, green and red light. Whole genome sequencing of the centric diatom *Thalassiosira pseudonana* indeed revealed the presence of putative

genes encoding cryptochromes and phytochromes. These photoreceptors are now being studied at the molecular and biochemical levels, and their function is being explored by reverse genetics approaches in the pennate diatom *Phaeodactylum tricoratum*. Furthermore, a cryptochrome gene (CRY1) has also been isolated from this diatom. We have found that cry1 protein levels increase upon exposure to light and that both mRNA and protein levels are strongly regulated by a circadian rhythm, which results in alternative splicing of CRY1 mRNA and increased protein levels during subjective days. Interestingly, we have also found that *P. tricoratum* cells are phototactic, moving towards blue light and away from red light. The photoreceptors involved in this process are currently being examined.

IL88

Synthesis and accumulation of mycosporine-like amino acids: an essential strategy to minimize UV damage in freshwater organisms

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Aquatic organisms have developed numerous strategies to minimize the accumulation of damage from exposure to solar UV radiation, however, not all strategies appear to be feasible or efficient at a certain time. For example, enzymatic repair may be constrained by suboptimal temperatures and the occurrence of photoprotective pigments may represent a trade-off when visual predators are present. Moreover, most photoprotective pigments are restricted to particular taxa. In contrast, non-colored sunscreen compounds such as mycosporine-like amino acids (MAAs) are widespread among different taxa and have been described for polar to tropical ecosystems. Our knowledge about the occurrence and photoprotective role of MAAs is largely based on studies of marine organisms. However, a substantial body of evidence has developed during the last years that shows that these sunscreen compounds are also widespread among freshwater organisms living in ecosystems of different UV transparency. This presentation will provide an overview of the accumulating evidence for the photoprotective role of MAAs and their ecological importance in different freshwater organisms.

IL89

Models and molecular markers for UV-B stress evaluation in aquatic ecosystems

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The determination of a universal quantitative parameter suitable to assess the UV-B induced radiative stress level in a biological system is still an open question, even if a large amount of data has been collected about the effects of UV-B on molecules, cells and organisms.

Another open, very important problem for global studies, is the assessment of how much an ecosystem is stressed by UV-B, the determination of the dynamic course of this stress (and of the associate risk) under an exposure to a UV-B radiation increasing in time. Data collected in the laboratory, in fact, are often difficult to relate to the effects taking place in a real ecosystem; on the other hand, most of the *in situ* studies deal with systems that are too complex to allow the development of useful, reliable models of the influence of UV-B on ecosystems.

In this work we have approached these two problems in two complementary ways, intended to bridge laboratory and *in situ* studies. The first approach consists in the set-up of laboratory-made microecosystems, which can allow the study of the dynamics of UV-induced damages in mathematically tractable ecosystems and the investigation of complex phenomena due to species-species

interactions. We discuss the results obtained by studying a hypersaline-type microecosystem.

The second approach is aimed at the investigation, by means of genomic and proteomic techniques, of the metabolic pathways influenced by UV-B radiation. The main goal of this approach is the identification of some molecular markers, specific for UV-B induced stress and suitable for a quantitative assay of the level of radiative stress in an organism. We are performing this investigation in some ciliates of different habitats (freshwater, marine, extremophile) searching for common UV-B induced metabolic pathways. On the basis of the obtained results, we discuss the feasibility of the design of DNA- and/or protein-chips suitable to measure UV-B stress level in real ecosystems.

OC90

Impact of solar UV radiation on bacterioplankton and phytoplankton biomasses and activities at different depths in NW Mediterranean coastal water during summer

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UV impact on bacterio- and phytoplankton have been repeatedly demonstrated for surface incubations, however depth distributions of these effects were poorly investigated. *In situ* experiments were conducted at 5 different depths (between 0 and 10 m) in oligotrophic NW Mediterranean coastal water to determine the impact of solar UVR upon bacterio- and phytoplankton. We measured changes in biomass and productivity for both communities under three solar radiation treatments: ambient radiation (PAR +UV), PAR only and dark, after one day of exposure and after night repair. We repeated this experiment on four occasions in June and July 2004. The effects of UVR were significant at the surface but decreased rapidly with depth. No significant effects could be measured at 5 m depth for most parameters. Picophytoplankton appeared to be more sensitive to UV compared to larger phytoplankton cells. Bacterioplankton recovered completely from protein synthesis inhibition due to UV radiation after the night repair period. Absolute levels of damage were relatively low when compared with results obtained at other locations. We supposed that natural populations of bacterio- and phytoplankton present in summer in Mediterranean Sea could be adapted to high UV fluxes.

IL91

The role of mitochondria in photoaging and aging

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Mitochondria are cell organelles that evolved from so called purplebacteria approximately 1.5 billion years ago. These organelles are responsible to supply the cell with the energy intermediates ATP through the respiratory chain located at the inner mitochondrial membrane. In close proximity to this lies the mitochondrion's own genome, the mitochondrial (mt) DNA. Mitochondrial DNA is a 16kb long, double stranded, circular molecule that exists in 4-10 copies per mitochondrion. Since the respiratory chain is one of the major generators of reactive oxygen species (ROS) the mtDNA is particularly exposed to oxidative stress. Therefore, mutations of mtDNA have been shown to accumulate during the normal aging process while the antioxidant capacity of a cell decreases during normal aging and only recently it has been shown in mice that these processes are causally linked to the normal aging process. We and others have previously shown that mtDNA mutations not only play a role in chronological aging but also in premature aging of the skin induced by chronic sun

exposure (photoaging). Mutations of mtDNA are increased in chronically sun exposed skin with clinical signs of photoaging and these mutations can be induced *in vitro* in normal human fibroblasts as well as *in vivo* in normal human skin by repetitive UV-exposure. In addition to this it could be shown that these mtDNA mutations do impair mitochondrial function and induce the expression of matrix metalloproteinase (MMP)-1. Since mutations of mtDNA persist for at least 18 months in human skin after cessation of irradiation these mutations do represent a long-term marker for UV-exposure of human skin.

IL92

Changes in elastin and microfibrillar component in photoaging

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Photoaged skin contains elastotic materials in the upper reticular dermis. This phenomenon is commonly known as solar elastosis. Little is known about the mechanisms leading to the accumulation of elastotic materials in photoaged skin. We demonstrated that ultraviolet radiation induced tropoelastin expression in the keratinocytes and fibroblasts of human skin *in vivo*, and that tropoelastin expression in photoaged skin was higher in the keratinocytes as well as in dermal fibroblasts, compared to intrinsically aged skin of same individuals. Heat is generated as a consequence of infrared irradiation, and leads to an increase in skin temperature during sun exposure. We demonstrated that heat increased tropoelastin expression in the epidermis and dermis of human skin. On the other hand, fibrillin-1 expression were increased by heat in the epidermis, but were decreased in the dermis. Ultraviolet light and heat induced MMP-12 in human skin *in vivo*. We also found that photoaged human skin expressed significant amounts of MMP-12 protein, which colocalized with the material of solar elastosis, whereas there was little expression in intrinsically aged skin of the same individuals. Therefore, our results suggest that the abnormal production of tropoelastin and fibrillin by ultraviolet light and heat in human skin and their degradation by various MMP, such as MMP-12, may contribute to the accumulation of elastotic material in photoaged skin.

IL93

Epidermal-dermal interactions in photoaging and photocarcinogenesis.

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Long term consequences of sun exposure are photoaging and cancer development, affecting the two major cutaneous compartments, dermis and epidermis respectively. Human skin reconstructed *in vitro*, comprising a fully differentiated epidermis and a living dermal equivalent is a useful tool to identify cell type specific biological damages, as well as their interactions. UVB is responsible for direct epidermal damage, i.e. DNA lesions and apoptotic sunburn cells. UVA can directly target the dermal compartment, inducing ROS generation and fibroblast alterations. Xeroderma pigmentosum (XP) is a rare recessive photosensitive syndrome due to impaired nucleotide excision repair of UV-induced mutagenic lesions. Clinical traits of XP (group C) disease lie in a high proneness to skin cancers in photo-exposed area and premature signs of skin aging. A DNA-repair deficient skin *in vitro* has been constructed using primary XP-C keratinocytes and fibroblasts, allowing to reproduce DNA-repair deficiency after UVB exposure. The presence of XP-C fibroblasts resulted in increased contraction of the dermal equivalent and provoked keratinocyte invaginations, indicative of early steps of neoplasia. MMP-1 is a major actor of skin response to UV exposure and participate in extracellular matrix remodelling. In reconstructed skin, its production is assumed by fibroblasts, directly when UV radiation reached the dermal compartment or indirectly through the

release of epidermal soluble mediators. We performed a fine characterisation of XP-C fibroblasts and found an aged-like phenotype with over-expression of MMP-1. Increased MMP-1 protein is associated with higher mRNA level resulting from up-regulation of the *MMP-1* gene transcription. These data show that reconstruction of skin *in vitro* is a valuable tool to study dermal-epidermal interactions, a crucial process in both photoaging and photocarcinogenesis.

IL94

Overexpression of a putative ubiquitin c-hydrolase isolated from PUVA-senesced fibroblasts results in a senescence-like phenotype

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Premature ageing of the skin is a prominent side effect of psoralen plus UVA (PUVA) photochemotherapy, used for various skin disorders. Following PUVA-treatment fibroblasts undergo long time growth arrest reminiscent of replicative senescence. Since the molecular basis of the functional and morphological changes is unknown, we applied subtractive hybridization to isolate genes that might be involved in this cell cycle arrest. Out of several differentially expressed cDNAs, one cDNA of an unknown gene showed the highest change with a 6-fold induction post PUVA-treatment. Using RT-PCR and RACE, we isolated a full length cDNA revealing a 53bp 5'-untranslated region, a 3222bp open reading frame and a 2269bp 3'-untranslated region. In addition, three transcripts with high homology were detected. Northern blot analysis revealed strong expression in postmitotic human tissues like brain and heart, but no expression in proliferating cells. From the translated cDNA sequence, the first 400 amino acids show high homology with a putative murine ubiquitin c-terminal hydrolase releasing ubiquitin from ubiquitinated proteins. Overexpression in fibroblasts resulted in a senescent phenotype with enlarged cell size, cessation of proliferation, expression of senescence-associated β -galactosidase and of matrix metalloproteinase-1, and high levels of reactive oxygen species. The de-ubiquitination enzymes are involved in biologically important processes including growth and differentiation, cell cycle progression, and signal transduction. Analysis of this unknown gene might be useful in understanding the role of de-ubiquitination in stress-induced and physiological senescence.

OC95

Free-electron laser photoelectron emission microscopy of human pigments

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The application of UV-free electron laser photoelectron emission microscopy (UV-FEL PEEM) to measure the threshold photoelectron spectrum and photoionization potential for human pigments is described. The origin of potential artifacts and the limitations of the technique are discussed and their potential effects on the measured photoionization potential are quantified. Analysis of the FEL-PEEM data revealed ionization thresholds of 4.6 and 3.9 eV corresponding to oxidation potentials of 20.2 and 10.5 V vs normal hydrogen electrode for eumelanosomes and pheomelanosomes, respectively. The difference in oxidation potential is attributed to the pigment content of the melanosome, namely whether it contains primarily eumelanin and pheomelanin. Age-dependent properties of pigments isolated from human retinal pigment epithelium cells will also be described. Changes are observed in the oxidation potential of human retinal melanosomes with increase age, suggestion oxidative damage to melanosomes with age may contribute to increased oxidative stress in the retinal

pigment epithelium. The oxidative properties of retinal lipofuscin granules and melanosomes as a function of age will also be presented.

OC96

Ultraviolet-B radiation inhibits local T cell activation and systemically induces T cell migration into the liver

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Ultraviolet B (UVB) radiation is known to be immunosuppressive and can induce a population of antigen-specific regulatory T cells in regional lymph nodes. However, the immunomodulatory effect of UVB on T cell activation is unknown. In a model of systemic UVB-induced suppression, we investigated the number of activated T cells in response to the contact sensitiser, oxazolone. C57BL/6 mice given 3 consecutive doses of UVB irradiation on their dorsal trunk were sensitised and challenged with oxazolone on their abdomen. One week following initial sensitisation and 24 hr post challenge, CD4 and CD8 T cells in regional and distal lymph nodes, spleen, peripheral blood and liver were assessed by 4-colour flow cytometry for status of activation. In the inguinal lymph nodes, which drain both the site of UVB irradiation and area of sensitisation, a decrease in the number of activated T cells (CD44hiCD62L-) was observed compared to unirradiated and sensitised mice. In addition, UVB in the absence of sensitisation also increased total and activated T cells in the draining nodes. The total number and percentage of activated T cells in mesenteric and cervical lymph nodes, as well as spleen, remained unchanged following treatment. Interestingly, UVB caused a decrease in the total number of T cells in the blood of either oxazolone sensitised or unsensitised mice. Moreover, increased numbers of activated and naïve (CD44loCD62L+) T cells were recovered from the livers of UVB irradiated mice, independently of whether they were sensitised, compared to untreated mice. In summary, UVB, in the absence of antigen, causes lymph node shut down and an accumulation of activated T cells in draining, but not non-draining lymph nodes and reduces the number of T cells activated by sensitisation. UVB also induces discrete rearrangements within the T cell pool to cause loss of T cells from the blood and migration into the liver.

OC97

Epidermodysplasia verruciformis human papillomavirus types are associated with squamous cell carcinomas of xeroderma pigmentosum patients

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Human papillomaviruses (HPVs) are common infections of the skin which are often found associated to benign and malignant skin lesions, especially the epidermodysplasia verruciformis (EV) HPVs, suggesting them to be involved in the development of non-melanoma skin cancer (NMSC). Xeroderma pigmentosum (XP), a rare autosomal recessive disorder deficient in DNA repair and hypersensitive to ultraviolet radiation, is remarkable for the high predisposition of very young patients to skin cancers. We have analysed 40 squamous cell carcinomas, 27 basal cell carcinomas and 9 normal skin biopsies from XP patients for presence of HPV DNA using a degenerate and nested polymerase chain reaction methodology. HPVs were detected more frequently in SCCs (20/40; 50%) than in BCCs (4/27; 15%) or normal skin biopsies (2/9; 22%). This association between HPVs and SCCs is limited to EV HPV types which were found in 48% of SCCs whereas

prevalence of cutaneous HPV types were only of 8% and no genital type was found. Moreover, the association between EV HPV and SCCs increases with age. The HPV spectrum characterised in XP lesions, includes 22 different EV HPV types, comparable to that found in NMSC from adult immunosuppressed organ transplant recipients. Our data, the first for skin cancers from young XP patients, show a statistically significant association between EV HPV and SCCs and underline the importance of HPV infection in skin carcinogenesis.

OC98

Examination of solar ultraviolet radiation exposure of road construction workers in Austria

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Ultraviolet radiation (UVR) is a proven carcinogen resulting in various forms of skin cancer.

To quantify UVR exposure of road construction industry workers involved in typical outdoor work, a study was conducted using UVR-sensitive polysulphone film badges and, additionally, electronic UVR-measurements. While conducting personal observation on site, information on the environmental, personal and work practice factors that affect personal UVR exposure was collected. A total of more than 1000 man day exposures, involving 37 workers, were measured between July and September at 50 different construction sites in the surrounding of Vienna (latitude: 48 °N).

A conversion factor was calculated giving the ratio between the measured erythemal radiant exposure at different parts of the body and the global erythemal radiant exposure. With this factor it was possible to extrapolate the erythemal radiant exposure for the workers for the whole year.

The results showed that the workers received an erythemal radiant exposure between 1000 J m⁻² and 1400 J m⁻² per day on roughly 100 days per year. On approximately 220 days the workers exposure exceeded 2 standard erythemal doses (SED) which are typical to induce UV-erythema in people of skin type I. Workers received more than 4.5 SED on 180 days, indicating that even workers of skin type IV are receive significant doses of UVR. Depending on the ground reflection and the reflection of the working materials, the recommended UV-A exposure limit for the eye was exceeded by up to a factor of 4.

An examination of the workers by a dermatologist following the measurements revealed many UV-induced skin lesions of different severity ranging from hyperpigmentation up to solar keratoses and one case of BCC.

In summary, the study found that road construction outdoor workers were exposed to high levels of UVR, in most cases without adequate sun protection.

OC99

Deficient inflammatory response in neonatal mouse skin may contribute to the susceptibility of HGF/SF transgenic mice to UV-induced melanoma

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UV irradiation has multiple effects on skin including inflammation, immunosuppression and the induction of keratinocyte-derived skin cancer and malignant melanoma. The current paradigm is that inflammation is protective against the initiation of cancer but is promotional in cancer progression via pro-angiogenic and anti-

apoptotic processes. We have derived a mouse model of UV-induced melanoma in which hepatocyte growth factor/scatter factor (HGF/SF) transgenic mice neonatally irradiated with UV produce melanomas which recapitulate human disease. In this model, while neonatal UV is sufficient for melanoma induction an additional adult dose of UV radiation significantly increased melanoma multiplicity.

We have investigated UV-induced inflammation in FVB wild type and HGF/SF transgenic mice. Mice were irradiated with an F40sunlamp (0 to 8.4 kJ/m²) at 3 days and/or at 21-25 days of age and skin taken 24-72h later. FACS analysis of freshly isolated skin cells stained with antibodies specific to granulocytes (Ly6G), macrophages (CD11b) or MHC class II was used to quantitate the inflammatory infiltrate which was confirmed by microscopic examination of skin sections.

In the present study, we show that the percentage of both granulocytes and macrophages significantly increased whereas cells positive for MHC class II decreased after UV irradiation in both wild type and HGF/SF transgenic adult mouse skin. Moreover the effect was time and dose dependent. In contrast no change of Ly6G⁺ or CD11b⁺ or MHC class II⁺ cells was detectable in neonatal mouse skin with any UV dose at any time point investigated, indicating major differences in the inflammatory response between neonates and adult. Application of trinitrochlorobenzene similarly initiated an inflammatory response in adult but not in neonatal skin.

The lack of inflammatory infiltrate in neonatal mouse skin may contribute to the susceptibility of neonates to melanoma induction by UV radiation.

OC100

Ultraviolet-induced ($\lambda=254\text{nm}$) degradation of the bacteriophage MS2 genome (ssRNA)

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It has not been possible up to now to routinely apply virologicals controls to drinking water because of the time-consuming nature of the gold standard technique (cell culture) and its lack of ability to detect all serotypes (e.g. *Norovirus*). Molecular techniques (e.g. real-time RT-PCR) constitute a solution to the rapid and specific detection of all the serotypes. However, ignorance of the mechanisms of viral degradation prevents the validation of PCR for the measurement of the risk of infection to humans following disinfection treatment.

This work was undertaken to gain an understanding of viral RNA degradation induced by ultraviolets (UV), which will be very useful in helping to define the significance of the presence of the viral genome in disinfected water. We focused our investigation on the influence of UV on naked RNA, encapsidated RNA and on infectivity. Our first results show that RNA damage (encapsidated or not) and viral inactivation are dose-dependent. The genome degradation seems related to the size and location of the detected fragment. Moreover, no capsid effect is observed since UV damage between naked RNA and encapsidated RNA. Our results also reveal a linear and rapid decrease in the infectious virus quantified by plaque forming units method. Comparison between plaque forming units method and real-time PCR (whatever the size and location of detected RNA is) for viral detection reveals disagreement following disinfection treatment.

The detection of genome fragments is insufficient to confirm the presence of the infectious virus, since each targeted fragment shows a different sensitivity.

OC101

Diastereoselectivity and site-dependency in the photochemistry of ketoprofen in the bovine serum albumin matrix

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A combined approach using global analysis of circular dichroism multiwavelength data and time resolved fluorescence was applied to investigate the interaction of R(-) and S(+)-ketoprofen with bovine serum albumin in buffer solution at neutral pH. A characterization of the most stable protein:drug adducts of 1:1 and 1:2 stoichiometry was obtained. The stability constants and the absolute circular dichroism spectra of the diastereomeric complexes were determined. A tryptophan residuum was shown to be involved in the binding of the drug, in the primary site for the R(-) and in the secondary site for the S(+) enantiomer.

The photodegradation of S(+)- and R(-)-ketoprofen (KP) enantiomers in the protein matrix (at molar ratios KP/BSA 1/1 and 2/1) was studied by steady state photolysis at $\lambda_{\text{irr}} > 320$ nm and transient absorption spectroscopy at $\lambda_{\text{exc}} = 355$ nm. The individual degradation quantum yields of the 1:1 and 2:1 KP:BSA complexes were estimated. R(-)-KP was found to be more labile than S(+). Two triplet ketoprofen species were evidenced for each optical antipode, with lifetimes depending on the chirality of the drug. Relative weights of the short and long lived components for each enantiomer depend on the drug/protein molar ratio. The triplet properties were rationalized on the basis of diastereoselective binding and site-dependent photoreactivity of the drug in the protein matrix.

IL102

Environment effects on the spectroscopy and intramolecular relaxation processes of C₆₀

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In this contribution, I will first discuss the environment effects on the fluorescence of C₆₀ trapped in rare gas matrices and in the pure solid. This will also demonstrate that in the latter case, the emission has a purely molecular character. The phosphorescence spectra of pure C₆₀ solid are also revisited and analysed in terms of emissions from electronically relaxed and unrelaxed triplet states. Finally, the ultrafast intramolecular energy relaxation processes in C₆₀ in toluene has been measured using a femtosecond fluorescence up-conversion experiment and the results will be discussed in relation to our previous work on C₆₀ in Ne and Ar matrices^{i,ii}.

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IL103

Organic and hybrid [60]-fullerene multicomponent architectures

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Owing to their unique electronic properties and structural features, [60]-fullerene and its functionalized derivatives are excellent building blocks for the construction of multicomponent arrays featuring photoinduced energy and electron transfer. Depending on the medium polarity or the specific design of the molecular

architecture, the C₆₀-type moiety can play the role of energy or electron acceptor. In the last number of yearsⁱ we have investigated the photophysics of several multicomponent arrays containing C₆₀ which have exhibited a variety of interesting photoproperties. Hybrids with Ru(II), Re(I) and Cu(I) coordination compounds^{ii-iv}, dyads and dumbbells with organic conjugated oligomers^{v-vii}, fullerodendrimers^{viii}, and fullerene-porphyrin arrays^{ix-x} have been studied. A brief survey of our work in this area will be given, highlighting the versatility of C₆₀ in the design of photoactive molecular architectures which may serve as active materials in photovoltaic devices, singlet oxygen sensitizers, charge separation centers, UV-VIS to NIR emission converters.

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We acknowledge EU for supporting our research in the field of fullerene photoscience (contract HPRN-CT-2002-00171, "FAMOUS")

IL104

Molecular engineering in artificial photosynthesis

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One of the most attractive strategies to construct organic solar cells and related photofunctional devices is to apply the basic principle operating in natural photosynthesis in terms of the conversion and storage of solar energy. We have prepared a variety of porphyrin–fullerene linked systems to mimic photoinduced energy transfer (EN) and electron transfer (ET) processes in photosynthesis. Photodynamical studies on porphyrin–fullerene linked systems have revealed the acceleration of photoinduced ET and charge-shift and the deceleration of charge recombination, which is reasonably explained by the small reorganization energies of ET in fullerenes. In particular, a ferrocene–porphyrin trimer–fullerene pentad revealed formation of a long-lived charge-separated state (0.53 s in frozen DMF at 163 K) with an extremely high quantum yield (83%), which is comparable to natural bacterial reaction centers. We have also constructed a variety of molecular assemblies of porphyrin as a donor and fullerene as an acceptor on electrodes for molecular photoelectrochemical devices. Highly efficient EN and ET processes have been realized at gold electrodes modified with self-assembled monolayers of porphyrin- or fullerene linked systems mimicking light-harvesting and charge separation in bacterial photosynthesis. Moreover, highly ordered organization of porphyrins and fullerenes has been achieved using step-by-step self-assembly of porphyrin and fullerene units by association with gold nanoparticles or dendrimers on tin oxide electrodes, which exhibit high power-conversion efficiency of up to 1.5%. These results will provide valuable information on the design of donor-acceptor type molecular assemblies that can be tailored to construct highly efficient organic solar cells. *Org. Biomol. Chem.*, 2, 1425 (2004); *J. Phys. Chem. B*, 108, 6130 (2004); *Chem. Eur. J.*, 10, 3184

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IL105

From photosynthesis to photonic molecular switches based on fullerenes

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Learning from photosynthesis, photochemists have designed and prepared artificial analogs of natural antennas and reaction centers. Photosynthetic reaction centers, which convert visible light into electrochemical potential energy through photoinduced electron transfer, have inspired the study of many covalently-linked assemblies of chromophores, electron donors and electron acceptors. It is now possible to construct artificial reaction centers that generate high-energy, long-lived charge-separated states in high quantum yield. Many of these employ fullerenes as electron acceptors because fullerenes demonstrate low reorganization energies for electron transfer and low sensitivity to solvent stabilization of the anion. Recently, we have been learning how to control such charge separation with light by using photochromic moieties, which can be isomerized back and forth between two structures using light of different wavelengths. By covalently linking a photochromic species to a chromophore, the photoisomerization reaction of the photochrome may be used to control photoinduced electron transfer involving the chromophore. Several approaches to such control have been investigated. For example, one isomer of a photochrome may be used to rapidly quench a porphyrin first excited singlet state by singlet-singlet energy transfer, precluding photoinduced electron transfer to an acceptor fullerene. Alternatively, the oxidation or reduction potential of a photochrome may be adjusted so that one isomer quenches an excited state of a nearby chromophore by photoinduced electron transfer, whereas the other isomer does not. Using such ideas, we have designed and studied molecular single- and double-throw switches and logic gates of various types that have photonic inputs and optical or electrical readouts.

IL106

Sequence-specific DNA photocleavage and potential applications

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Targeting of specific genes in a living organism is important both for scientific research and for therapeutical applications. For example, the treatment of cancers or genetic and viral diseases could be achieved by correction of expression of certain genes. The modulation of gene expression at genomic DNA level is the surest way for silencing of undesirable genes. DNA photocleavage is one of the widely used DNA modification methods. The advantage of photosensitizers is the possibility to start the treatment by light irradiation at a specific moment after the agent reached its target. An ideal photo-reagent must recognize specifically a targeted DNA sequence, then its excitation must be induced by using light which is not absorbed by tissues and blood. However, majority of chemical and photo-reagents are not sequence-specific. Thus, it is necessary to conjugate them with "addressing" molecules able to recognize specific DNA sequences.

Besides natural peptides, two classes of synthetic molecules are known to distinguish and bind specifically to double-stranded DNA sequences: triple helix-forming oligonucleotides (TFO) and oligo(N-methylpyrrole/N-methylimidazole) carboxamide minor groove binders (MGB).

In this presentation the following subjects will be reviewed:

1. Photosensitizers used for DNA modification and mechanisms of DNA photocleavage. The most popular categories of compounds: metal complexes and organic functional derivatives including porphyrins and fullerenes.
2. Sequence specificity of TFO and MGB.
3. Construction of sequence-specific photo-reagents. Three components of sequence-specific agents: "addressing" moiety, linker and photosensitizer. Methods of functionalization and conjugation of these components, with special attention to fullerene functionalization and conjugation to oligonucleotides.
4. Delivery of targeted agents into the living cells.
5. Recent advances in the development of DNA-photocleavage agents.

IL107

Radical scavenging by C₆₀ in model systems and inhibition by C₆₀ of hepatotoxicity induced by CCl₄ *in vivo*

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Radicals R[•] generated by photolysis, flash photolysis, and pulse radiolysis have been shown to react with one or more of the 30 carbon double bonds of [60]fullerene, also called C₆₀. For example, benzyl and methyl radicals can produce radical and non-radical adducts R_nC₆₀ with n reaching 15 to 34 respectively. Hepatotoxicity by carbon tetrachloride, a classical model system, is caused by metabolic activation leading to radical intermediates: CCl₃[•] and CCl₃O₂[•]. Recently, we have shown that pre-treatment with C₆₀ resulted in a dose-dependent prevention of acute liver injury in CCl₄-treated rats. The powerful liver-protective efficacy of C₆₀ against CCl₄ toxicity is probably due to the unique radical scavenging property of C₆₀ leading to non toxic addition products of CCl₃[•] and CCl₃O₂[•]. Alternatively, C₆₀ might also act as cytochrome P 450 inhibitor of CCl₄ metabolic activation. The possible mechanisms of protection including the effects of C₆₀ on Kupffer cells activation are under investigation in our laboratory.

IL108

Microbial rhodopsins: structure and mechanism in photomotility

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Microbial rhodopsins are a large family of photoactive membrane proteins found in both prokaryotic and eukaryotic microorganisms. We are studying the mechanisms of members of this family to understand how their common protein design, consisting of seven transmembrane helices forming a membrane-embedded pocket for the chromophore retinal, has been adapted to different physiological functions. Some are light-driven ion pumps ("transport rhodopsins"), whereas others are photosensory receptors ("sensory rhodopsins"). The sensory rhodopsins use various signaling mechanisms, including interaction with membrane-embedded transducers to control a phosphorylation cascade in haloarchaeal photomotility responses, interaction with a soluble cytoplasmic transducer in cyanobacteria, and control of calcium channels in Chlamydomonas photomovement. The systems in which most progress has been made on atomic structure/function are sensory rhodopsins I and II (SRI and SRII), phototaxis receptors that bind to transducers (Htr's) in haloarchaeal species. Crystallography of domains of the SRII-HtrII complex, Förster resonance energy transfer between sites on SRs and their cognate Htr's, peptide binding studies, time-resolved FTIR, and EPR spin-labeling work are beginning to give a moving picture of the signaling process. Tight interactions between membrane-embedded domains hold the cytoplasmic domains of the SR and Htr subunits in close juxtaposition, and SR photoactivation, which causes an

outward displacement of the cytoplasmic end of helix F that in turn changes the structure of the E-F loop, alters the interaction in the membrane-proximal region. We will present our current efforts to elucidate the molecular events in the photosignal transduction processes by microbial sensory rhodopsins.

IL109

Roles for photoactivated adenylyl cyclase (PAC) in photocontrol of flagellar movement in euglenoid cells

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Photoactivated adenylyl cyclase (PAC) was first purified from a photosensing organelle (the paraflagellar body) of the unicellular flagellate *Euglena gracilis*, and was shown to be the photoreceptor for the step-up photophobic response^{i,ii}. To further understand its role, kinetic properties of photoactivation of PAC was studied as well as the change in intracellular cAMP levels upon blue light irradiationⁱⁱⁱ: Activation of PAC was dependent both on photon fluence rate and duration of irradiation, between which reciprocity held well. Repetitive pulse irradiation of L:D=1:1 caused activation of PAC in a photon fluence-dependent manner irrespective of the duration of light pulses (down to 50ms). Wavelength dependency of PAC activation was in general accordance to the UV-B to visible absorption spectrum of its chromophore, FAD. The time course of the transient increase in intracellular cAMP level induced by blue-light irradiation corresponded well with that of the step-up photophobic response. Suppression of PAC expression by RNAi cancelled the increase in intracellular cAMP upon blue light irradiation. These results indicate that the transient increase in intracellular cAMP level evoked by photoactivation of PAC is actually a key event of the step-up photophobic response. Next, high-speed video analyses of the flagellar and cell movements in step-up photophobic response revealed that length of latent period showed photon fluence rate-dependency in agreement with that of PAC activation, indicating that accumulation of certain amount of the photo-produced cAMP is necessary for the change of flagellar movement. Downstream signal transduction will also be discussed based on additional observations on euglenoid cells.

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IL110

Phototaxis and signal transduction in the cyanobacterium *Synechocystis* sp PCC6803

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Motility in the unicellular gliding cyanobacterium *Synechocystis* sp. PCC6803 requires Type IV pili. Phototaxis appears to be a complex and regulated light-directed phenomenon in which single cells and groups of cells participateⁱ. To study signal transduction during phototaxis we have isolated several mutants with an aberrant phototactic response (i.e they are non-motile or exhibit constitutive negative phototaxis). Several of these mutants mapped to *che*-like genesⁱⁱ. *Synechocystis* sp has three loci containing *che*-like genes; all three of these (*tax1*, *tax2* and *tax3* loci) appear to play a role in phototaxis. Mutants in the *tax1* and *tax2* locus are negatively phototactic while mutants in *tax3* are non-motile. Our analysis of some of these mutants using a novel slide-based phototaxis assay and time-lapse video microscopy suggests that there are at least two light inputs that regulate phototaxisⁱⁱⁱ. There is a red light (far red

light reversible) positive phototaxis controlled by TaxD1 which fits with evidence that there are two phytochrome-like domains in TaxD1. These results suggest that while positive phototaxis is controlled by red light, negative phototaxis in *Synechocystis* sp. strain PCC6803 is mediated by one or more (as yet) unidentified photoreceptors. Analyses of specific site-directed mutants in the hybrid histidine kinase, TaxAY1, one of the key players in the signal transduction events, will be discussed. We will also describe some of the novel mutants that were isolated in the mutagenesis screen. Our working model of the complex signal transduction networks that control phototactic movement will be presented.

ⁱ Bhaya D Light matters: phototaxis and signal transduction in unicellular cyanobacteria *Mol Microbiol.* 53(3):745-54 (2004)

ⁱⁱ Bhaya D, Takahashi A, Grossman AR. Light regulation of type IV pilus-dependent motility by chemosensor-like elements in *Synechocystis* PCC6803 *Proc Natl Acad Sci U S A.* 98(13):7540-5 (2001)

ⁱⁱⁱ Ng WO, Grossman AR, Bhaya D. Multiple light inputs control phototaxis in *Synechocystis* sp. strain PCC6803. *J Bacteriol.* 185(5):1599-607 (2003)

IL111

A balanced purple-bacterial motility response to illumination

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Purple bacteria are attracted by light that can be used for photosynthesis. In addition, some genera are repelled by high-intensity and/or short-wavelength irradiation. In the last decade several studies have tried to gain insight into this 'blue-light' motility response. In *Halorhodospira halophila* at least one of the two identified photoactive yellow proteins is involved. *Rhodobacter sphaeroides* has a single *pyp* gene, and also shows a blue-light repellent response. However, deletion studies showed that its PYP protein is not involved in this response, and therefore an alternative photoreceptor remains to be characterized. The blue light response in *R.sphaeroides* is however complex and unpredictable. Individual cells subjected to an intense flash of blue light often respond by stopping while colonies usually move away from blue light. However, the response is inconsistent, suggesting that it may depend on other factors, such as growth conditions. This is different from responses measured to actinic red or white light, where a reduction in light intensity consistently leads to stopping and to colonies moving toward the light.

Besides *pyp* and *appA*, the genome of *R.sphaeroides* contains several additional genes that display homology to photoreceptor domains: Rsp4111 and Rsp4191 (bPHY domains), Rsp4060 and Rsp1261 (BLUF domains) and Rsp2228 (LOV domains). Based on the genomic context of Rsp2228 (next to putative Che proteins), we consider this a candidate for blue light sensing. However, deletion of neither the putative BLUF domain nor this complete BLUF/Che region resulted in a clearly observable phenotype.

We have recently isolated a number of chemotactic, photosynthetic but non-phototactic mutants. These will be screened for detailed responses to blue- and red light.

R.sphaeroides also forms substantial biofilms under micro-aerophilic, illuminated conditions. It is possible that light sensing mechanisms are involved in maintaining the integrity of these biofilms.

OC112

Signal perception and response in benthic diatom photomovement

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Biraphid diatoms from estuarine intertidal biofilms exhibit motile responses to light stimuli. These responses are strongly dependant on the intensity and wavelength of the light. The diatoms *Cylindrotheca closterium* and *Navicula perminuta* have been used as test organisms to examine the various types of photomovement. Both species were found to exhibit photokinesis, but only *N. perminuta* displayed a directional response to light gradients. Pronounced photokinesis was seen with red light as well as blue, whereas the directional response was only seen at wavelengths below 510nm. When high intensity blue light is applied to *N. perminuta* cells they stop, and then reverse direction. This type of photophobic response to a sudden increase in light intensity has been linked to changes in intracellular calcium concentration in some motile algae. To investigate the photophobic response in more detail we have developed a routine method for monitoring cytoplasmic Ca²⁺ in *N. perminuta* and *C. closterium* which allows simultaneous stimulation with high intensity blue light and fluorescence measurements of an intracellular calcium indicator. The results indicate a role for cytoplasmic Ca²⁺ in the direction reversal response.

PL113

Oxidative DNA damage: from electron/hole injection to gel electrophoresis

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The photosensitized oxidation of DNA by Type I mechanisms involving the initial one-electron oxidation of nucleobases has been extensively investigated. The sites of oxidation, especially guanines, have mostly been studied by gel electrophoresis techniques that are highly sensitive and allow for one-nucleotide resolution of the DNA damage. Much valuable information has been gained by these methods. Recently, the applications of mass spectrometric methods has led to a deeper understanding of the nature of the DNA photo-oxidation products, especially those resulting from the reactions of guanine. On the other hand, applications of spectroscopic laser pulse-induced electron transfer and free radical reactions in our laboratory have led to novel insights into the initiation phases of the complex, multi-stage photo-oxidative reactions in DNA. However, the complex sequence of events that are initiated by the one-electron oxidation of DNA that culminate in specific guanine oxidation products, are still not well understood. A combination of transient absorption spectroscopy, high resolution gel electrophoresis, and the separation and identification of oxidation products by reversed phase HPLC and mass spectrometry techniques, are providing new opportunities for understanding the complex phenomena associated with the photosensitized oxidation of DNA.

IL114

UVB and UVA wavelengths target different skin compartments: implications for photo-immunology

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Visible consequences of acute or chronic sun exposure vary from short term sunburn reaction to long term effects such as UV-induced skin cancers and photoaging. In parallel, photo-immunosuppression also occurred, leading to decrease in local or systemic immune reactions. It is now well admitted that both UVB and UVA rays are involved in these deleterious effects. However, these wavelengths domains act through different molecular mechanisms; i) direct DNA lesions for UVB and ii) generation of

reactive oxygen species for UVA. In addition, the high penetration properties of UVA led them reach the deep layers of the skin. The human skin model *in vitro* represents a valuable tool to reproduce typical markers of a sunburn reaction. DNA lesions, P53 accumulation and apoptotic keratinocytes could be found in epidermis after UVB exposure. In contrast UVA alterations were mostly located within the dermal compartment with ROS generation, commitment of fibroblasts into apoptosis and direct MMP-1 production. These UVA effects induced by single or repetitive exposures have been associated to photoaging process. Release of several cytokines (IL1 α , IL6, IL8) could also be found from both cell types and depends on the wavelength domain. Release of keratinocytes derived IL1 α and IL6 after UVB exposure are involved in dermal MMP-1 production by fibroblasts. Finally, the integration of Langerhans cells in reconstructed epidermis allowed to mimick the impact of UV radiation on this cell type. The data illustrated the use of reconstructed skin models for determination of early cellular and molecular targets induced by the two UV wavelengths domains as well as their spatial distribution, which may be crucial for events related to photoimmunosuppression.

IL115

A role for Platelet Activating Factor receptor binding in UV-induced immune suppression and skin cancer induction

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The UV radiation found in sunlight is the primary cause of non-melanoma skin cancer and is implicated in the induction of malignant melanoma. Exposure to UV radiation is also immune suppressive. UV-induced immune suppression is a major risk factor for sunlight-induced skin carcinogenesis. UV-exposure activates a cytokine cascaded involving PGE₂, IL-4 and IL-10 that induces immune suppression. However, the earliest molecular events that occur immediately after UV exposure, especially those upstream of PGE₂ secretion, are not well understood. Within minutes of UV exposure, UV-irradiated keratinocytes release the lipid mediator of inflammation, platelet-activating factor (PAF). UV-irradiation up-regulates the transcription of the cyclooxygenase-2 gene, leading to the secretion of PGE₂, and the transcription of IL-10. Treating keratinocytes with PAF-receptor antagonists blocks both events, suggesting a role for PAF in activating the cytokine cascade. Treating mice with PAF or UV suppressed delayed type hypersensitivity *in vivo*, and injecting mice with PAF-receptor antagonists blocked both PAF and UV-induced immune suppression. In addition, treating UV-irradiated mice with PAF-receptor antagonists suppressed skin cancer induction. These studies identify a novel role for lipid mediators of inflammation, the induction of immune suppression, and suggest a novel use for PAF receptor antagonists in blocking skin cancer induction.

IL116

The osmolyte taurine plays a critical role in ultraviolet B radiation-induced immunosuppression

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We have previously shown that osmolytes such as taurine are part of the stress response of human keratinocytes to ultraviolet B (UVB) radiation (Warskulat et al., J. Invest. Dermatol., 2004). Accordingly, UVB induces the expression of different osmolyte transporters in longterm cultured human normal keratinocytes (HNKs). The expression of the transporter for taurine (TAUT) is highly inducible in UVB exposed HNKs and leads to increased

uptake of osmolytes, especially taurine. Moreover, taurine uptake protects human skin cells against UVB-induced effects. Preloading of HNK *in vitro* with taurine prevents both hyperosmotic stress and UVB-induced gene expression of TNF- α and IL-10. We therefore speculated that taurine uptake might be involved in skin protection against UVB-induced immunosuppression. To test this, we assessed UVB-induced immunosuppression using the delayed type hypersensitivity assay in TAUT knock-out mice, which have more than a 90% reduction of taurine levels in their skin. Comparative studies of the immune response revealed that homozygous, but not heterozygous TAUT knock-out mice were significantly more susceptible towards UVB-induced immunosuppression than wildtype mice. Platelet activating factor (PAF), which can be generated from cell membrane phospholipids, has previously been shown to mediate UVB-induced immunosuppression (Walterscheid et al., J. Exp. Med., 2002). Since taurine can strongly bind to phospholipids, we wondered whether increased susceptibility of TAUT knock-out mice towards UVB was detectable at the level of PAF production. We found that treatment of TAUT knock-out mice with a PAF receptor antagonist overcomes their increased sensitivity towards UVB-induced immunosuppression. PAF itself, however, induced immunosuppression, irrespective of the expression of TAUT. These results indicate that lack in taurine results in elevated release of PAF or PAF-like lipids from the cell membrane upon UVB exposure. Taken together these studies suggest that taurine uptake – similar to DNA repair and pigmentation – is critically involved in endogenous photoprotection of skin cells against UVB radiation-induced detrimental effects.

IL117

UVA induced immunosuppression

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It is well established that ultraviolet radiation has immunomodulatory effects which may be involved in skin cancer. Recent studies have shown that UVA radiation (320-400 nm) as well as UVB (290-320 nm) is immunosuppressive. This means that sunscreens which mainly absorb UVB (protection against erythema) may be less effective in preventing UVR-induced immunosuppression than broadspectrum products. We have studied the effects of UVA exposure on the human delayed-type hypersensitivity response (DTH) to recall antigens and compared the efficacy of sunscreens having different levels of UVA protection under both solar simulated radiation (SSR) chronic exposures or acute exposure and outdoor real-life solar exposure conditions. Contact hypersensitivity reaction (CHS) to DNCB was also used to compare the protective efficacy of two sunscreen products under acute SSR exposure.

Our studies clearly demonstrate the role of UVA in the induction of photoimmunosuppression together with the need for sunscreen products providing efficient photoprotection throughout the entire UV spectrum. These data suggest that sun protection factor (SPF) may not be sufficient to predict the ability of sunscreens to protect from UV-induced immune suppression. Determining the level of UVA protection is particularly needed since UVA seems to have a relatively low contribution to erythema but is highly involved in immunosuppression.

OC118

UV-A-induced immunosuppression plays a role in UV-A-induced melanoma metastasis in mice

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The possibility that UV radiation may affect tumor metastasis has been addressed by a limited number of studies, which have focused on the immunosuppressive effects of UV-B radiation. We have previously shown *in vitro* that UV-A enhances the metastatic properties of melanoma cells by increasing the adhesiveness of B16-F1 melanoma cells to endothelium and changing expression of

adhesion molecules. We have also shown *in vivo* that UV-A increases the formation of melanoma lung metastases in C57BL/6 mice injected *i.v.* with B16-F1 cells. The aim of the present animal study was to confirm the previously observed *in vivo* UV-A effect and to determine whether UV-A-induced immunosuppression might be one of the mechanisms responsible for it. Obtained results have confirmed that mice, that were *i.v.* injected with B16-F1 cells and exposed to UV-A, develop 14 days after treatment 4-times more of lung metastases as compared with the non-exposed group. The *in vitro* exposure of melanoma cells, prior to injection into mice, has lead to induction of 1.5-times more metastases as compared with the animals injected with non-irradiated cells. This suggests that the UV-A-induced changes in the adhesive properties of melanoma cells do not, alone, account for the 4-fold metastasis increase. However, the UV-A dose (8 J/cm²) used in this study was found to cause systemic immunosuppression determined by contact hypersensitivity (CHS) assay using oxazolone as the sensitizing agent. Therefore, we suggest that the extent of UV-A-induced increase of metastasis *in vivo* might be a combination of the UV-A-induced change in melanoma adhesiveness and the UV-A-induced weakened immunity.

OC119

5-HT₂ blockage prevents psoralen+UVA (PUVA)-induced suppression of delayed hypersensitivity

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PUVA has been used for many years as a very effective treatment modality for various skin diseases, including psoriasis and cutaneous T cell lymphoma. PUVA-induced immune suppression may be the mechanism by which PUVA exhibits therapeutic effects and leads to clearance of skin lesions. However, the molecular events by which PUVA leads to immune suppression are not well understood. Activation of serotonin (5-hydroxytryptamine, 5-HT) receptors may be one event involved in PUVA-induced immune suppression. In this study we used the 5-HT₂ receptor antagonists ketanserin and cyproheptadine to study the possible role of 5-HT signaling in PUVA-induced systemic immune suppression in the model of delayed-type hypersensitivity (DTH) to *Candida albicans*. Six to 8 wk old C3H/HeNcr mice were painted on their shaved backs with 8-methoxypsoralen (100 ug/100 ul ethanol) 30 min before exposure to 50 kJ/m² UVA (i.e. minimal inflammatory PUVA dose, as determined by skin swelling measurements 48 h after exposure). At day 5, the mice were immunized by injecting 2 x 10⁸ formalin-fixed *C. albicans* into each flank, and at day 14 they were challenged by injecting 50 ul of *Candida* antigen into each hind footpad. PUVA suppressed the immune response in the mice by approximately 80%. The intraperitoneal injection of either ketanserin or cyproheptadine (100 ul of 5mM solution in 50% DMSO each) immediately before UVA exposure nearly totally abrogated PUVA-induced immune suppression. These results suggest that 5-HT signaling is involved in PUVA-induced immune suppression. A better understanding of the molecular events after PUVA exposure may offer the opportunity to dissect the beneficial (i.e. therapeutic) from the detrimental (i.e. tumorigenic) effects of this treatment modality.

OC120

Ultraviolet radiation-induced immunosuppression in humans is prevented by topical nicotinamide (vitamin B3) and is greater in men than women

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We investigated the effects of topical nicotinamide on UV-induced suppression of delayed type hypersensitivity (DTH) responses to tuberculin purified protein derivative (PPD) in healthy, Mantoux positive volunteers. Twenty volunteers were exposed to graded doses of solar-simulated (ss) UV radiation on their backs daily for 3d. Adjacent, unirradiated areas served as immunologically intact controls. Before each irradiation, 5% nicotinamide or base lotion was applied to various sites on the back in a double-blinded manner. PPD was injected at each of the irradiated and unirradiated sites after the final UV exposure and the intensity of the resulting Mantoux reactions was measured 72h later with an erythemameter. We found that DTH responses at base lotion-treated sites were suppressed by 20 – 44% when subjects were exposed to low dose ssUV equivalent to 3 to 9 minutes of Sydney summer sunlight. Application of nicotinamide before UV exposure prevented UV immunosuppression at all doses. Nicotinamide did not function as a sunscreen as it had no effect on the minimal erythema dose, and again prevented immunosuppression when applied after instead of before UV exposure in another 20 volunteers. We also studied the effect of gender on susceptibility to UV immunosuppression in our volunteers. Men are known to have higher skin cancer incidence and mortality and we found that men were significantly immunosuppressed at ssUV doses 3 times lower than those required to immunosuppress women. Hence the higher skin cancer risk of men may be at least partly due to impaired immunity. We conclude that nicotinamide is a non-toxic compound which could be added to sunscreens to help prevent UV immunosuppression in humans. Nicotinamide is an inhibitor of the DNA-repair enzyme poly (ADP-ribose) polymerase and a precursor of the coenzyme NAD⁺, required for intracellular respiration. We suggest that depletion of nicotinamide and its metabolites and PARP inhibition may be mechanisms of photoimmunosuppression.

OC121

DX5+ NKT cells from UV-irradiated mice regulate tumor immunity by suppressing CLT activity

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The ultraviolet radiation present in sunlight plays a critical role in the initiation and promotion of non-melanoma skin cancer because of its DNA-damage and immunosuppressive properties. Previously we reported that UV-induced immune suppression is mediated by NKT cells, which can transfer immune suppression to non-irradiated normal recipients. We hypothesized that suppression was due to NKT cell-mediated inhibition of cytotoxic T cell (CTL) activity. To confirm this hypothesis, suppressor NKT cells were generated by chronically irradiating C3H mice with 15 kJ/m² of UV radiation, three times a week for 12 weeks. Splenic, CD4+/DX5+ and CD4+/DX5- cells were purified by magnetic microbeads. *In vivo*, 2x10⁶ purified CD4+/DX5+ or CD4+/DX5- T cells were transferred to non-irradiated syngeneic recipients via tail vein injection and the mice sensitized to the UV-2240 tumor cell line via intra-dermal injection. After 14 days, splenocytes were isolated and cultured for 4 days for use in a CTL assay. Four days later CTL assay was performed to see if DX5+ NKT cells could inhibit CTL-activity. In a separate *in vitro* experiment purified splenic CD4+/DX5+ or CD4+/DX5- T cells were cultured for 3 days in 96-well-plates coated with anti-CD3 antibody, to activate them. The cells were then washed and co-cultured with UV-2240 *in vivo* primed mice splenocytes for 4 days. CTL assay was performed

to see if *in vitro* activated DX5+ NKT cells could inhibit CTL-activity. Flow cytometry analysis showed that the CD4+/DX5+ cells were positive for CD3, CD44 and partially positive for CD62L and V 8.1, 8.2 but negative for V 2 and Ly49A. Both the *in vivo* and *in vitro* CTL assays showed that CD4+/DX5+ but not CD4+/DX5- cells significantly suppressed UV-2240 target cell killing compared to controls. Our results identify the mechanism by which DX5+ NKT cells mediate UV-induced immune suppression and it involves inhibition of CTL activity and function.

OC122

Molecular changes in skin induced by exposure to different doses of ultraviolet A radiation

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Ultraviolet (UV) wavelengths in sunlight have been found to cause skin cancer by suppressing the immune response and causing genetic damage to cells within the skin. The UVA waveband has immunomodulatory effects although the molecular mechanisms remain to be elucidated. We recently demonstrated in C57BL/6 mice that 1500 mJ/cm² of UVA caused immunosuppression while 750 mJ/cm² enhanced secondary immunity and 3000 mJ/cm² was immunoprotective. These doses are equivalent to 3-10 minutes of exposure to summer sunlight in Sydney. The aim of this project was to compare the molecular events occurring in response to these doses of UVA. A microarray study comparing UVA irradiated and non-irradiated C57BL/6 mice was used to compare the regulation of biological pathways by 750, 1500 and 3000 mJ/cm² of UVA. The results from this study identified key biological pathways including, MAPK signalling, complement cascade, prostaglandin signalling and Glutathione signalling, which were differentially regulated by the three doses UVA. Immunoenhancing (750 mJ/cm²) and immunoprotective (2990 mJ/cm²) UVA doses caused the most changes with an opposing regulation of many genes in the MAPK signalling pathway. The immunosuppressive (1500 mJ/cm²) UVA dose reduced expression of genes involved in complement activation and greatly increased the expression of COX-2, a key member of the prostaglandin signalling pathway. In the glutathione signalling pathway, the immunosuppressive dose caused an opposing regulation of many genes in comparison to the immunoenhancing and immunoprotective doses. Some of the molecular changes identified from this microarray study, such as the regulation of COX-2, have previously been well documented to be involved in immunomodulation. These findings indicate that UVA causes a complex dose related effect on many biological pathways with different doses having opposite effects.

OC123

Photoimmunoprotection by UVA (320-400 nm) radiation is determined by UVA dose and is dependent on cutaneous cyclic guanosine monophosphate (cGMP)

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The immune modulating properties of the UVA waveband have been controversial. Here we demonstrate in the hairless mouse that single sub-inflammatory UVA radiation exposures between 1.61 to 580.5 kJ/m² do not systemically suppress the contact hypersensitivity (CHS) reaction. Furthermore, a window of UVA doses was defined between 16.13 to 580.5 kJ/m² that increasingly provided immunoprotection against UVB-induced suppression of CHS. Higher UVA exposures (870.8 to 1161 kJ/m²) caused increasing mild oedematous skin inflammation, became immunosuppressive alone, and lost the immunoprotective capacity against UVB radiation. We have previously reported that UVA photoimmunoprotection depends on the UVA induction of

cutaneous heme oxygenase-1, particularly its product, gaseous carbon monoxide (CO). The CO was suggested to activate cutaneous guanylyl cyclase, as topical application of the specific guanylyl cyclase inhibitor, ODQ, abrogated UVA photoimmunoprotection in the mouse. This study shows that the cutaneous guanylyl cyclase is responsive to UVA, but was activated only by the immunoprotective UVA doses, or by immunoprotective treatment with topical CO. Its product cGMP increased for 48 h after an immunoprotective UVA exposure. However, cGMP concentration was reduced by inhibition of guanylyl cyclase with ODQ, and conversely was increased by inhibition of its degradation by phosphodiesterase (PDE) activity with sildenafil. The PDE-5 isoform mRNA was identified in normal mouse skin. Subsequently we found that topical sildenafil at a moderate concentration protected effectively against suppression of CHS by solar simulated UV radiation (SSUVR) or the immunosuppressive UVB photoproduct *cis*-urocanic acid. Thus cutaneous cGMP, controlled by its synthesis via CO-activated guanylyl cyclase and its degradation by PDE-5, is a strong determinant of UVA photoimmunoprotection.

IL124

Optical diagnosis and treatment in hollow organs

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Optical techniques are suited for detection of lesions that are not visible to the naked eye or which are difficult to distinguish from each other. These include mucosal dysplasia which can develop in the epithelium all hollow organs, particularly the mouth, major airways, gastrointestinal tract and urogenital tract. Elastic scattering spectroscopy can be used to detect dysplasia as an 'optical biopsy'. Photodynamic diagnosis using ALA esters has been particularly successful in the bladder.

Photodynamic therapy (PDT) is attractive in hollow organs as it does not affect the collagen scaffold on which the organ is built. This means that the structural integrity is maintained whilst the tissue is undergoing repair after treatment. Examples of successful implementation of PDT include treatment for dysplasia and early cancer in Barrett's oesophagus and the airways using Photofrin and in the oral cavity using Foscan. Both these drugs have recently received licences. 5-amino laevulinic acid is still under investigation for treating dysplasia in Barrett's oesophagus, but looks very promising. This drug is particularly exciting for superficial lesions as it does not carry the risk of oesophageal stricturing or prolonged skin photosensitivity that Photofrin does, although for deeper lesions, it is not effective. Palliation of advanced bile duct cancer has also been very successful with Photofrin and large randomised controlled trials are under way.

Conclusion: the success of translational research in this area is resulting in new, effective treatment for many previously difficult to manage conditions.

IL125

Photodynamic therapy for cancers of the head and neck

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Head and neck cancers constitute a unique category of human malignancy. Traditional treatment modalities carry significant functional and aesthetic impairment leading to withdrawal and social isolation. The management of head and neck cancers is further complicated by the occurrence of multiple primary malignancies. Treatment of a second primary cancer within the previously operated or irradiated field is even more difficult. The difficulties associated with treating these tumors suggest a need for

alternative treatments that are less destructive, repeatable, and compatible with previous and subsequent radiotherapy and surgery. Photodynamic therapy (PDT) is a non-thermal reaction, the necrosis is localized and healing takes place with little scarring, and good preservation of function. Surgery, radiotherapy, or chemotherapy does not preclude the use of PDT, nor will PDT compromise the subsequent use of any other treatment. Results from previous studies have shown that PDT is of similar efficacy as traditional measures in the treatment of early-stage head and neck cancers with an overall response rate of 85%-100%. For advanced head-and-neck cancers, studies showed that 58%-70% palliative benefit can be observed in these patients. The survival of the patients can be further improved by the use of interstitial PDT. Good treatment responses were obtained and long-term survivors were occasionally encountered. In addition to good treatment outcomes, the cost-effectiveness of PDT is also superior to palliative chemotherapy and extensive palliative surgery. Recent developments in biochemical and molecular technology have improved the specificity and efficacy of photosensitizers. PDT is a therapeutic option that may prove a useful addition to the armamentarium of the integrated head and neck oncology team.

IL126

Photodynamic diagnosis and photodynamic therapy for brain tumors

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Introduction: the median survival of patients suffering from malignant brain tumors is 12 months despite all available therapy. Photodynamic applications, such as Photodynamic Therapy (PDT) and most recently Photodynamic Diagnosis (PDD) are currently undergoing intensive clinical investigations as adjunctive treatment modality for malignant brain tumors. Survival is directly related to the extent of surgical resection. We developed a system for intraoperative tumor detection and fluorescence guided resection which facilitates orientation and allows a more radical tumor removal employing mTHPC. This was followed by intraoperative photodynamic therapy.

Over 510 patients were treated worldwide by PDT for malignant brain tumors following tumor resection in various open clinical phase I/II trials, which includes 85 patients at the own institution. Photosensitisation was performed in most of the patients by Fotofrin/ Photosan as well as by second generation sensitizer mTHPC. Intraoperative fluorescence was induced by a UV light source at 370-440nm. A standard neurosurgical microscope was optimized for fluorescence detection. Intraoperative PDT was carried out by lasers at the appropriate wavelengths and energy.

Results and Conclusion: variations in the treatment protocols, photosensitizers and light dose make the evaluation statistically difficult, however there is a clear trend towards prolonging median survival after one single photodynamic treatment as compared to standard therapeutic regimens. Primary glioblastomas multiforme WHO IV and recurrences demonstrate a median survival of 22 and 9 months. Other entities such as metastasis, skull base tumors or pituitary tumors responded completely in the majority of the cases. PDD proved to be useful in tumor resection accounting for a radical resection in 75% under fluorescence guided resection as compared to 30% in historical group.

PDT was generally well tolerated and side effects consisted of increased intracranial pressure and prolonged skin sensitivity against direct sunlight.

In addition to our own experience on PDT and PDD mediated by first and second generation photosensitizers we will give an overview of the general results and highlighten the potential future application of photodynamic application in neurosurgery.

IL127

Review of interstitial photodynamic therapy for tumours of solid organs

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Lesions that are too deep for PDT with superficial illumination can be treated interstitially by inserting laser fibres through needles positioned percutaneously under image guidance. Experimentally, interstitial PDT (iPDT) can produce necrosis in a range of organs without damage to the mechanical or functional integrity of surrounding tissues and with safe healing. Preliminary clinical data are available for several cancers. 16 patients with inoperable, localised cancers of the head of the pancreas were treated with iPDT. Tumour necrosis was documented in all (median survival 12 months, range 4-36 months, compared with 6-9 months in historical controls) with no treatment related mortality. Complications were seen (gastrointestinal bleeds and duodenal obstruction), but were treatable. 13 patients with localised recurrence after radiotherapy for prostate cancer were treated. The prostate specific antigen (PSA) fell in 9 (to undetectable levels in 2), 5 had no evidence of cancer on post iPDT biopsies. There were fewer complications than after conventional salvage therapy (surgery or hormones). In 39 patients with advanced head & neck cancers, worthwhile responses were seen in 32 including complete responses in 9. iPDT can produce several cm of necrosis in the pig lung, which heals safely with preservation of the major airways and blood vessels passing through the treated zone. This makes it a therapeutic option for small peripheral lung cancers in patients unfit for conventional therapy. iPDT may be applicable in other solid organs such as the brain, kidney or thyroid.

Conclusion. iPDT is well tolerated and can achieve worthwhile palliation with low morbidity and mortality in a range of cancers unsuitable for conventional treatment, with occasional long term survivors in those with a presumed poor prognosis.

OC128

Synergistic combination of photochemical treatment and bleomycin on tumor growth

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Objectives. Photochemical internalisation (PCI) is under development for improving macromolecular therapy by inducing photochemical damage to endocytic vesicles. PCI induces damage to endocytic vesicles leading to release of therapeutic macromolecules entrapped in endocytic vesicles into the cytosol. The photosensitizer disulfonated aluminium phthalocyanine (AIPcS_{2a}) has previously been utilized experimentally for this purpose *in vivo* to enhance the therapeutic potential of the protein toxin gelonin. Bleomycin is used in several standard cancer chemotherapy regimens, inducing single- and double-strand DNA breaks. Its hydrophilic and relatively large chemical structure limits its ability to penetrate membrane structures which causes accumulation into endocytic vesicles. The purpose of this study has been to evaluate the therapeutic potential of combining AIPcS_{2a}-based photochemical treatment and treatment with bleomycin.

Methods. Three subcutaneously growing tumors of different origin were selected. AIPcS_{2a} and bleomycin were systemically administered and the tumor area exposed to red light. The tumors were exposed to light when the tumor volume had reached 100 mm³. Tumor volume was measured frequently after treatment and the time for the tumor volume to reach 800-1000 mm³ was selected as the end point.

Results. The combined treatments induced an increased regrowth time and in 2 out of three tumor models 50-60 % complete response. Combination of the two treatment modalities was found by statistical analyses to induce a synergistic delay in tumor growth.

Conclusion. AIPcS_{2a}-based PCI of bleomycin induces a synergistic effect on growth delay and cure in 3 different tumor models. This

combination treatment should be further considered for clinical utilization.

OC129

Evaluation of the photosensitizer Tookad[®] for photodynamic therapy on the Syrian golden hamster cheek pouch model: light dose, drug dose and drug-light interval effects

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The response to photodynamic therapy (PDT) with the photosensitizer Tookad[®] was measured in the Syrian hamster cheek pouch model on normal mucosae and chemically induced squamous cell carcinoma. This photosensitizer is a palladium-bacteriopheophorbide presenting absorption peaks at 538 and 762 nm. The light dose, drug dose and drug injection-light irradiation times (DLI), ranging between 100-300 J/cm², 1-5 mg/kg and 10 to 240 min respectively, were varied and the response to PDT was analyzed by staging the macroscopic response and by the histological examination of the sections of the irradiated cheek pouch. A fast time decay of the tissular response with drug dose of 1 to 5 mg/kg was observed for DLI ranging from 10 to 240 min and for light doses of 100-300 J/cm² delivered at a light dose rate of 150 mW/cm². A significantly higher level of tissular response was observed for squamous cell carcinoma compared to normal tissue. Nevertheless, the threshold level of the drug-light dose for a detectable response was not significantly different in the tumoral versus normal tissue. The highest response at the shortest DLIs and the absence of measurable response at DLI larger than 240 min at light dose of 300 J/cm² and drug dose of 5 mg/kg reveals the predominantly vascular effect of Tookad[®]. This observation suggests that Tookad[®] could be effective in PDT of vascularized lesions or pathologies associated with the proliferation of neovessels.

OC130

Photodynamic therapy and fluorescent diagnostics in head and neck cancer patients with different photosensitisers

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Photodynamic Therapy (PDT) and fluorescent diagnostics (FD) with Photosense was done in 50 patients with head and neck cancer (HNC) and in 89 patients with skin cancer, using Radaclorin (RC) in 42 patients with basal cell carcinoma (BCC), in 6 patients with oral cancer, FD with Alasense (AS) in 127 patients with BCC, squamous cell carcinoma (SCC). Detecting of the borders of tumor, intensity of accumulation of photosensitisers in tumor, normal tissues, visualization was done by Spectral-fluorescent Complex (He-Ne-laser). We've got 2-dimensional pictures of fluorescence of all tumors using AS: in 52% of patients it exceeded the borders of clinically detected ones and the intensity of fluorescence in SCC was positively higher than in BCC. In 35,7% BCC patients additional fluorescence zones were found, cytological verification - in 93,3%. We've got fluorescence of all tumors using PS and RC, additional fluorescence zones were found, cytological verification of BCC was got. We used semiconductive lasers for PDT: Milon - 660, Biospec- 672. Multiple laser surface and interstitial irradiation was performed 24 hours after PS injection with total light dose till 400-600 J/cm² and single light irradiation with light dose 200 - 300 J/cm² using RC. 2 months after PDT with PS in HNC we've had complete response (CR) in 66.0% and partial response (PR) in 30.0% of patients. 2 months after PDT with RC in 42 BCC patients there was ORR -100% with CR in patients with BCC T1-2NOMO - 92.9%, in patients with recurrences of cancer CR - 60,6%, PR - 39,4%. The efficacy of PDT with PS in BCC was higher (CR - 86.7%, PR - 13,3%) and the recurrence rate in 6 months lower in

patients with T3-4 stage BCC. Our experience show pronounced efficacy of PDT for head and neck tumors of different localization and histology. FD provided significant information about disease advance, allowed identification of subclinical lesions, demonstrated high sensitivity and specificity.

OC131

Photodynamic applications of superficial bladder cancer : from detection to treatment!

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A photodynamic application resulting from the interaction of a photosensitizer and a specific light leads to either a fluorescence response and/or a cell-death mechanism within a selective tissue. In Urology, fluorescence cystoscopy induced by aminolevulinic acid derivatives such as HAL (hexaminolevulinic) is accepted as a user-friendly new diagnostic tool in the endoscopic management of bladder tumors.

Its counterpart, photodynamic therapy or the induction of cytotoxic mechanism in cancer tissue that is based on the same photosensitizing agents but with different conditions of illumination remain experimental. However, recent results raise great hopes of new therapeutic directions.

IL133

Light signalling and plant transcriptome patterns

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Plant growth and development are strongly affected by light signals. Light signals are fluctuations of the environment that carry information about conditions that require adjustment of the form and physiology of the plant body. Some fluctuations of the light environment can be informative in a given developmental or physiological context (plant age, plant tissue, set of reactions) and non informative in a different context. Plants are therefore posed with the evolutionary challenge imposed by the simultaneous need to respond to some of the changes in the light environment (signals) and ignore others to attain both plasticity and robustness.

The information provided by the analysis of the transcriptome in plants of *Arabidopsis thaliana* exposed to different light conditions is helping to uncover the patterns of reaction to changes in the light environment at the genomic level. We are using near full genome *Arabidopsis thaliana* microarrays in combination with single and multiple photoreceptor mutants and specific light treatments to investigate the role played by different photoreceptors and their interactions in the generation of these patterns. We will describe study cases where light perceived by the phytochrome family of photoreceptors mediate unexpected light responses of the transcriptome and other cases where members of the cryptochrome and/or phytochrome family of photoreceptors actually alleviate light responses of the *Arabidopsis thaliana* transcriptome.

The complex responses of the transcriptome result from the components and the architecture of the light signalling circuitry. A genetic follow-up of the microarray experiments is helping to uncover these features of the signalling network and recent advances will be presented.

IL134**Quantitative trait locus analysis of the phase of the *Arabidopsis* circadian clock**

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The circadian clock is a major adaptation to the rotation of the earth, which results in daily rhythms of light and temperature. Mutant screens have identified a number of putative components, but an alternative approach using forward genetics takes advantage of natural allelic variation in *Arabidopsis* ecotypes to identify areas of genome responsible for variation in clock traits. The circadian parameter we are primarily interested in is 'phase', i.e. the timing of a point of the circadian cycle relative to an external timing cue (or 'zeitgeber'). It is the primary circadian target of natural selection. We have previously observed natural variation in phase in different accessions of *Arabidopsis* and wished to determine the loci controlling this trait. We use a transgenic approach to monitor circadian output, measuring the activity of the *cab2::luc*⁺ transgene in *Arabidopsis* seedlings, this being the first time that transcriptional activity has been used for a quantitative trait study in plants. We chose the timing of the peak of *cab2::luc*⁺ expression after the last zeitgeber (i.e. dawn) as our phase marker. Phase was measured in *Arabidopsis* seedlings from a population of recombinant inbred lines (RILs), derived from two parental genotypes (*Ler* x *Cvi*). Quantitative trait locus (QTL) analysis attempts to correlate phenotypic variation with genetic variation within a population; both types of variation arise from parental natural allelic variation. We found loci in which *Cvi* alleles either advanced or delayed the phase relative to *Ler*. Three loci were confirmed in near isogenic lines (NILs). One locus (chromosome 1) is not linked to any genes previously implicated in the circadian system, and represents a novel circadian locus. Another on chromosome 5 contained two genes known to affect clock function, *PRR3* and *SRR1*. We found previously unknown polymorphisms in both genes, indicating they are key members of pathways entraining the clock.

IL135**A GAF-domain mutation of phytochrome a impairs chromophore incorporation and light responses in *Arabidopsis***

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The plant photoreceptor phytochrome A (phyA) modulates plant growth and development in response to far-red light (FR) by two modes of action: the very low fluence response (VLFR), which is saturated at very low fluences of red or far-red light, and the high irradiance response (HIR). Here we report an *Arabidopsis* mutant (*phyA-303*) impaired in HIR but not in VLFR. Genetic complementation identified it as a phyA mutant, which carries a unique mutation in the conserved Arg384 present in the GAF domain of the protein (R384K). Feeding seedlings of the mutant plants with biliverdin partially rescued the *phyA-303* phenotype. *In vitro* studies with the recombinant, mutated protein resulted in a remarkably reduced expression yield of the apo-protein in such a way that further studies had to be performed with its identically mutated ortholog, phytochrome A from oat. Also in oat phyA, the R384K mutation led to a strong reduction of the expression yield, and an even lower yield of chromophore incorporation into this remaining protein amount. To ensure that the impaired functions of the mutated phytochromes were due to only a single amino acid exchange, and not been caused by improper expression protocols, the originally present arginine was placed back into the mutated

protein. This "rescued" recombinant phytochrome showed perfect and wild-type characteristic assembly kinetics and spectral properties. Apparently, a single mutation in the GAF (=chromophore-binding) domain reduces strongly the assembly of a functional phytochrome, most probably by impairing the correct folding into the functional three-dimensional conformation. It is suggested that signaling of phyA, to raise the VLFR and the HIR takes place via two different pathways of protein-protein interactions, for which two different regions of the phytochrome molecule offer contact sites, one of them (HIR pathway) being absent or strongly impaired by the single point mutation identified here.

IL136**Low-dose UVB induces a p53-dependent gene program that increases the resilience of keratinocytes against future UVB-insults**

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One protein central in the response of human keratinocytes to ultraviolet B damage is p53. By transactivating genes involved in either cell cycle arrest or DNA repair, p53 has a leading role in the recovery from this damage. Considering this role, we wished to investigate whether the triggering of a p53-dependent gene program by repetitive UVB-exposure can induce an adaptive response in human skin cells. In particular, we examined two p53-target genes, p21/WAF1 and p53R2, with a crucial role in p53-induced cell cycle arrest and p53-induced DNA repair respectively. Exposure to a mild UVB-dose was able to induce an adaptive response in human keratinocytes, leading to increased survival of cells that maintain their capacity to repair DNA damage upon exposure to apoptotic doses of UVB. Our study indicates that this adaptation response is only achieved if the interval between subsequent UVB-insults allows sufficient time for the p53-induced protective gene program to be induced. Our results also demonstrate that small but quickly recurring UVB-exposures are as harmful as one intense, continual exposure to ultraviolet B irradiation. Thus, provided there is enough time to recover, a gradual exposure to mild levels of UVB can increase the resistance of skin cells via the upregulation of p53 target genes, involved in growth arrest and repair. Together with other adaptation mechanisms at cellular level, such as heat shock protein induction, and at the tissue level, such as tanning and epidermal thickening, which also require time, the skin may become more resistant against acute damaging effects of UV. However, further research is warranted in order to explore alternative ways to activate a protective adaptive response without causing the damage that induces it in the first place. Hence this p53-dependent gene program could become a potential therapeutic target to test actives which increase the own resilience of skin cells against UVB damage.

IL137**Heat shock proteins in intrinsic photoprotection**

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All organisms respond to sudden environmental changes with the increased transcription of genes belonging to the family of heat shock proteins (hsp). Hsp-inducing stress factors include elevated temperatures, alcohol, heavy metals, oxidants, and agents leading to protein denaturation. The induction of heat shock proteins is followed by a transient state of increased resistance to further stress and the heat shock response is generally thought to represent an evolutionary conserved adaptive mechanism to potentially hostile and rapidly changing environmental conditions. Hsp are expressed in resting as well as in stressed epidermal and dermal cells and overexpression of hsp is associated with increased resistance to cell

death induced by UVA and UVB. The removal of UVB-induced cyclobutyl pyrimidine dimers is enhanced immediately after heat shock in keratinocytes, melanocytes, and fibroblast. On the other hand, no influence of heat shock on UVA-related oxidative damage could be demonstrated so far. Repeated exposure of keratinocytes in culture to heat shock or hsp-inducing agents does not lead to adaptation or attenuation of the response. Furthermore, aging does not result in a deterioration of the ability to express and induce hsp72 in human skin. From these and other results it has been concluded that hsp in the skin might provide an adaptive cellular response to UV and that it might be possible to utilize hsp-inducing agents as a new way to deal with the immediate and long-term consequences of UV-exposure. Prerequisite for the utilization of this concept is the development of non-toxic heat shock inducers and their evaluation for clinical efficacy and safety.

IL138

Biological effects of simulated ultraviolet daylight – New approach to investigate daily photo protection

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The irradiance of standard UV daylight is representative of environmental light exposure conditions and can be used to investigate the biological effects of a non-extreme UV light.

The simulated UV daylight used in this study is characterized by a UVA to UVB irradiance ratio of 24, while this ratio for the simulated zenithal UV light (UV-SSR) is close to 10.

The aim of our studies was to compare biological effects induced, in human skin, by acute or semi-chronic exposure to simulated UV daylight and to compare the efficacy of this non-extreme UV light with the one of simulated zenithal UV light (UV-SSR).

The results were expressed both according to the same biological doses based on erythema (MED) and according to identical physical doses.

Differences between biological efficacies of simulated UV daylight and UV-SSR indicate that the spectral distribution of the UV spectrum is of primary importance when dealing with biological effects induced in the epidermis (SBC, p53, CPD, Langerhans cells and melanocytes alterations...) and in the dermis (collagen, tenascin...) by UV light.

Interestingly, significant biological damage was noticed after 19 exposures to 0.5 MED of UV daylight spread over 4 weeks. This dose (0.5 MED = 7.6 ± 1.4 J/cm² UVA+UVB) is a very low dose of UV daylight, corresponding to 1/9 of the daily typical dose available in Paris in April (68 J/cm² of UV daylight between 6 a.m to 8 p.m); this emphasizes the importance of a good daily UV protection.

In conclusion, simulated UV daylight is a relevant new approach for daily photoprotection studies.

IL139

Effects of repeated sub-erythral UVR exposure on human skin *in vivo* and the role of sunscreens in their prevention

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There are few data on repeated sub-erythral exposure on human skin. Such exposure is likely to have long-term consequences so we studied the effects of 11 consecutive sub-erythral (~ 0.5MED) exposures to solar simulating radiation (SSR) in sun-sensitive skin types I/II (n = 6) as well as the photoprotective properties of a broad-spectrum (***) low SPF (7.5) sunscreen (6% Parsol SLX + 2% Parsol 1789) in comparison with a vehicle SSR control and a no irradiation control. We estimated that the daily SSR dose received through the sunscreen treated site was less than 0.1MED. Erythema was assessed daily and immuno-markers of proliferation, molecular and cellular damage were assessed, from skin sections, immediately after irradiation on days 5 and 11, and on day 12 which was 24 hours after the final irradiation. The data show that the protocol

induced erythema, DNA photodamage (thymine dimers), p53 protein expression and Langerhans cell depletion, against which the sunscreen offered significant or virtually total protection. There was no evidence of enhanced epidermal proliferation with any treatment and, surprisingly, no sunburn (apoptotic) cells were observed, even in the vehicle control group. The sunscreen protected against DNA damage but this still occurred and accumulated with an estimated daily dose of less than 0.1 MED. These data suggest that a "daily care" sunscreen may offer protection against non-intentional sub-erythral solar exposure.

OC140

Cosmesis-relevant biophysical changes in Asian volunteers induced by extensive exposure to sub-erythral doses of ultraviolet daylight. Influence of sun filters and textiles

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Until recently, Asians traditionally minimized extensive sun exposure by staying out of the sun, or by wearing suitable clothing. Yet, changing live-styles and clothing habits make traditional means often non-practicable. Hence, UV-filters integrated in daily care creams are evolving as alternative. Since appropriate reduction also of UV-A exposure has been demonstrated time and again in Caucasians to prevent photo-aging, proper UV-A protection may have similar effects also in Non-Caucasians.

The effect of long-term UV-exposure on skin color and cosmetic conditions of conventional (UPF 4) and of protection-augmented (UPF 13) textile, and of a day cream with comparable UV-B (15±1% UV-B transmission) and increasing UV-A protection (40, 30, 20% UV-A transmission) were assessed on the back of 22 Asian volunteers (Siamese or Philippine origin). Test areas were exposed for 12 weeks 3x/w to 0.8±0.1MED (solar light simulator), and skin color, skin moisture, skin profile and skin elasticity assessed prior to treatment and every 4 weeks.

Indeed, prolonged exposure to UV-light a) reduced skin moisture, b) increased skin wrinkles and reduced smoothness, and c) increased skin lifting and reduced firmness also in Asians in a dose-dependent manner. Such photo-damage was reduced but not prevented by an ordinary T-shirt, and even more so by protection-enhanced cloth, or by the cream with moderate UV-A protection. Yet, only creams with stronger UV-A protection prevented photo-damage and even improved the status of the skin, suggesting a synergistic influence of cream base and UV-A-protection.

OC141

Sunscreen use related to UV exposure, age, sex and occupation based on personal dosimeter readings and sun behaviour diaries

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Objective: to examine during which behaviour people apply sunscreen and to assess the relation to UV exposure monitored by personal dosimetry and diaries.

Participants: a convenience sample of 340 Danish volunteers: children, adolescents, indoor workers, sun worshippers, golfers and gardeners (age range, 4-68 years).

Measurements: subjects recorded sunscreen use and sun exposure behaviour in diaries and carried personal, electronic UV dosimeters, measuring time-stamped UV doses continuously in median 119 days covering 346 sun-years (1 sun-year equals 1 subject participating during 1 summer-season).

Results: there were great variations in sunscreen use, which was highly correlated to risk behaviour (sunbathing/exposing upper body) (r=0.39; P<.01). Sunscreens were used in median 5 days per sun-year, (range: gardeners 1 day - sun worshippers 16 days). 10% females and 41% males never used sunscreens. Females used sunscreens more but had also more unprotected risk behaviour than

males (8 days vs. 4 days, $P < .001$). Sunscreen use was not correlated to age and children had as much unprotected risk behaviour as adults. Sunscreens were used 86% of the days with risk behaviour in Southern Europe vs. 20% in Northern ($P < .001$). The UV doses were significantly higher on days with sunscreen ($P < .03$) and on sunburn days ($P < .001$). The median sun protection factor (SPF) was 10.5. The sun protecting effect corresponded to an application density of 0.5 mg/cm².

Conclusions: days with sunscreen did not correlate with days without risk behaviour but with days "sunbathing with the intention to tan" indicating that sunscreens was used as tanning aids to avoid sunburn during risk behaviour.

IL142

An outbreak of photoallergy

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Photoallergic contact dermatitis (PACD) is thought to be an uncommon event that arises when UV irradiation interacts with a hapten or antigen producing a photoexposed site dermatitis response.

A wide range of responsible chemicals have been reported; currently sunscreens and topical non-steroidal anti-inflammatory agents are particularly important.

Photopatch testing is the investigation of choice. Throughout Europe a range of methodologies have been used and published. In recognition of the importance of harmonisation, a group of interested European Contact Dermatologists/Photobiologists met to produce a consensus methodology statement, which extended to test materials and interpretation of photopatch testing. This publication¹ is essentially a preamble for a Pan-European standardised methodology photopatch test study.

Topical non-steroidal anti-inflammatory agents are tested to varying degrees throughout Europe. A recent outbreak occurred of contact and photoallergic dermatitis amongst workers packaging Rimadyl® (Carprofen) a Cox-2 inhibitor (for veterinary use). Limited exposure to the drug in an industrial setting was enough to produce sensitisation. The clinical cases and the results of investigations on five patients and controls will be produced.

Carprofen appears an extremely potent photosensitive agent. The possibility of pet owners developing photosensitisation should be borne in mind.

Reference: ¹ The European Taskforce for Photopatch Testing. Photopatch testing: a consensus methodology for Europe. Journal of the European Academy of Dermatology and Venereology 2004; 18: 679-682.

IL143

Drug-nucleoside interactions in the excited states: a model to investigate the basic mechanisms of drug-photosensitized DNA damage

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Photoinduced DNA damage frequently involves sensitization mechanisms, as direct sunlight absorption by the chromophores present in its pyrimidine and purine bases is relatively inefficient. In this context, drugs may play an important role as photosensitizers, absorbing the otherwise inactive UVA fraction of solar radiation and mediating both formation of pyrimidine cyclobutane dimers and guanine oxidation.

In the present work, the molecular mechanisms of drug-photosensitized damage to nucleic acids have been investigated by examining the photoreactivity of nonsteroidal anti-inflammatory drugs (i. e., ketoprofen) towards the key DNA constituents (thymidine and deoxyguanosine). The involved processes have been studied at the intermolecular level, with drug/nucleoside mixtures, but also at the intramolecular level, using covalently linked dyads containing the two units. Steady-state photolysis, followed by preparative HPLC and structural elucidation by

spectroscopic methods, has been performed to gain some insight into the nature of the primary photochemical processes and to establish the structures of the final products. On the other hand, time resolved experiments (basically laser flash photolysis) have been carried out to detect the responsible excited states, quantify their reactivity and achieve the direct observation of any possible transient reaction intermediates.

As there is an increasing trend to use chiral drugs in their enantiomerically pure active forms (rather than racemates), the above mentioned studies have been carried out in parallel with the (*S*)- and (*R*)-forms of the drugs. The results have provided evidence for a significant stereodifferentiation in the relevant excited state interactions.

IL144

Spectrum of cross-photosensitization in patients with contact photoallergy to ketoprofen: associated photoallergies to non-benzophenone-containing molecules

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Contact photoallergy to ketoprofen gels has been widely reported, and cross-sensitivity reactions with other compounds, such as tiaprofenic acid, fenofibrate, and benzophenones are well known. However, positive photopatch tests to other different non-benzophenone-related compounds have recently been observed. We report the results of photopatch testing in patients with contact photoallergy to ketoprofen and discuss the spectrum of cross-sensitization to ketoprofen. As expected, we observed positive photopatch tests to Ketum* gel and ketoprofen 2.5 % in petrolatum in all patients (100 %), to fenofibrate (78%), to tiaprofenic acid (61%), to benzophenone-3 (33%), and to fragrance mix (83%). However, it was remarkable to note positive photopatch tests to other unexpected and non-relevant allergens, including fenchlor (67 %), tetrachlorosalicylanilide (28 %), triclosan (17 %), tribromsalan (11 %), and bithionol (11 %), with no clinical relevance. Interestingly, these agents belong to the family of halogenated salicylanilides and related compounds, which have been forbidden in Europe since the 1970s. Furthermore, when tested with components of fragrance mix, 85% to 100% patients positive to KP reacted to cinnamic aldehyde or cinnamic aldehyde. Several hypothesis have been suggested to explain the high photoreactivity linked to ketoprofen. The role of aldehyde group near a benzene ring or the association of a benzene ring with an oxygen group could induce sensitization and cross-reactions between KP and different compounds. Indeed, several molecules, including KP, cinnamic alcohol, cinnamic aldehyde, benzophenone-3, fenofibrate, tiaprofenic acid, and several unexpected and non-relevant allergens share this chemical combination and are involved in cross-sensitization. Based on the strong correlation between sensitization to cinnamic alcohol and KP, we suggested that cutaneous reactivity to cinnamic alcohol may represent a risk factor of KP sensitization. Furthermore, exposure to the sun may facilitate the direct transformation making sensitization and cross-sensitization occur more rapidly. For this reason, we recommend to inform patients with positive tests to fragrance mix and cinnamic alcohol/aldehyde of the risk of cross-reactivity to KP.

IL145

Identification of photoallergic potential in drugs and other chemicals

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Photocontact allergy is a delayed-type hypersensitivity, mediated via the formation of a protein-photoallergen conjugate that acts as a complete antigen.

In order to behave as a photoallergen, a chemical must be able to absorb light of a wavelength present in sunlight; upon light absorption, the chemical must then generate reactive species, such as free radicals, capable of reacting covalently with proteins present in the skin. A simple procedure employing UV spectroscopy has been developed to test the ability of chemicals to form covalent protein conjugates after irradiation with the appropriate wavelength of light.

A number of investigations have been carried out into the relationships between the structural features of chemicals and their ability to exhibit photoallergy, most recently as part of European Community BIOMED research project BMH4-97-2590.

Structural rules for the prospective identification of new or untested photoallergens have been derived for a number of different chemical types, e.g. halogenated aromatic compounds, diaryl ketones, sulphonamides and aromatic nitro compounds (M.D. Barratt (2004) Structure–Activity Relationships and Prediction of the Phototoxicity and Phototoxic Potential of New Drugs, *ATLA* 32, 511–524). The main criterion for the generation of a structure-activity rule is that it should be based on a sound mechanistic rationale leading to the biological activity.

Relationships between the structure and properties of chemicals can be programmed into knowledge-based computer systems such as DEREK for Windows (DEREK is an acronym for “Deductive Estimation of Risk from Existing Knowledge”).

OC146

Photochemical internalisation of EGFR-targeting toxin conjugates

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Photochemical internalisation (PCI) is a novel modality for the cytosolic release of macromolecules and chemotherapeutics from endocytic compartments. Site-specific light activation of endocytic vesicle-localising photosensitizers results in photochemical membrane damage of the organelles and subsequently cytosolic release of the drugs. Specific tumour drug delivery can be achieved by conjugation of the macromolecule of interest to a targeting molecule, e.g. antibody/antibody fragment or a growth factor. In the present study we report on the effect of PCI of the type I ribosome-inactivating protein saporin conjugated to either epidermal growth factor (EGF) or to the chimeric monoclonal antibody cetuximab (C225/Erbitux®). The EGFR-targeting conjugates were made by coupling EGF-biotin/cetuximab-biotin to saporin-streptavidin. The toxicity of the conjugates was evaluated in a reticulocyte lysate assay and on cells by the MTT cell survival assay. Selective binding and internalization by EGFR was evaluated in three different ways; (i) Toxicity of the conjugates was measured in both EGFR positive and EGFR negative cell lines. (ii) Fluorescence microscopy of Alexa488 labelled conjugates was applied in the absent and presence of competing EGF or cetuximab. (iii) Antibodies against EGFR were used to inhibit the effect of PCI of the toxin conjugates. The present findings show that the targeting conjugates are internalised via EGFR in two different cell lines and exert high toxicity after PCI. PCI of EGFR-targeting toxin conjugates may be a potent anti-cancer therapy approach and warrant further work with *in vivo* preclinical models.

OC147

Primary processes in the photochemistry of fluoroquinolones

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Some of fluoroquinolones with potent anti-bacterial activity used in the treatment of bacterial infectious diseases are known to induce photosensitised reactions. It has been suggested that their mode of action be via production of excited singlet oxygen and other

reactive oxygen species in many cases, as well as due to the formation of photohaptens.

We have investigated photophysical properties of a number of quinolones and determined singlet oxygen yield for four of them. Steady-state fluorescence measurements were used to determine the pK_a^* of the first excited singlet state. Laser flash photolysis and pulse radiolysis have been used to study the excited states and the free radicals of the fluoroquinolones in aqueous solution. These compounds were found to undergo monophotonic photoionisation from the excited singlet state. Quantum yield for this process and the extinction coefficient of the cation radical formed were also determined. The extinction coefficient of the cation radicals were confirmed by pulse radiolysis experiments, where the oxidation was carried out with one electron oxidants such as Br_2^- . Other photophysical parameters such as triplet-triplet absorption spectra, intersystem crossing efficiency, triplet life-time, excited singlet oxygen formation etc. have also been determined. Mechanisms for primary photochemical reactions and the implication in photosensitivity of these compounds will be discussed in detail.

OC148

In vitro phototoxicity of lovastatin and a selected group of vitamin D3 isomers

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The phototoxic potentials of 7-dehydrocholesterol, cholecalciferol and Lovastatin were tested individually and in combination using our validated 3T3 NRU assay. The rationale was based on (1) the interrelated biosynthetic pathways for Vitamin D and cholesterol, and (2) the known hypersensitivity to ultraviolet radiation associated with hypocholesterolemia in man (Smith-Lemli-Opitz Syndrome). Only 7-Dehydrocholesterol was phototoxic by itself (PIF value 2.5). Of the combinations tested, Lovastatin significantly enhanced the 7-Dehydrocholesterol phototoxicity (PIF value 5.1), while Cholecalciferol neither enhanced nor decreased the phototoxicity of 7-Dehydrocholesterol. The mechanism(s) responsible for the observed phototoxicity were not determined. While 7-Dehydrocholesterol is a photochemically active compound, Lovastatin (a cholesterol-lowering pharmaceutical) is not considered photochemically active. However, other investigators have demonstrated increased sensitivity to long-wave ultraviolet radiation (i.e., UV-A) in cell cultures exposed to enough “statin” compound to lower membrane cholesterol. While the *in vitro* screen may be used to identify individual items of potential photobiological interest, pre-clinical (and possibly clinical) testing will be needed to determine whether pharmaceuticals that influence cutaneous cholesterol metabolism carry any photobiological risk under actual use conditions (or whether this illustrates another disparity between *in vitro* and *in vivo* test systems).

OC149

Carbanion-mediated photocages: from drug photostability to carbanion kinetics and new photocages

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The prompt generation of carbanions within the duration of a nanosecond laser pulse in the photochemistry of ketoprofen (a modified benzophenone) provides a way of evaluating absolute rate constants for carbanion reactions. Molecules can be designed so that they can undergo typical nucleophilic substitution and elimination reactions, processes that frequently compete with protonation of the carbanion.

Studies of intramolecular carbanion-mediated elimination reactions provide a new entry into molecular photocages, allowing the efficient release or simple groups (alcohols, halides, phosphates) and molecules of biological interest; the photorelease of ibuprofen

will be used to illustrate this case. The modification of the benzophenone chromophore to achieve improved absorbance in the UVA region will also be discussed.

Pelliccioli and Wirz (*Photochem. Photobiol. Sci.*, 2002, 1, 441.) outlined the six most important criteria that an ideal photocage would meet in the context of biological applications; they include: 1) clean and efficient photochemistry, 2) adequate absorption at wavelengths longer than 300 nm, 3) good aqueous solubility, 4) generation of inert and non-absorbing photoproducts, 5) high decaying rate constant, and 6) absence of 'dark' activity. The ketoprofenate chromophore satisfies all six criteria and is a significant step forward in the evolution of photocaging chromophores.

IL150

Plants in cold environments: an introduction to the symposium

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Plants occur in a range of extreme environments: extreme heat *versus* extreme cold, extreme aridity *versus* aquatic environments, chemically pristine *versus* polluted and highly saline environments, high solar irradiance *versus* dense shade, high UV-B regimes in high tropical mountains *versus* low UV-B regimes in the Arctic, stability on rock surfaces *versus* frost heave in soils. Many of these extreme environments will persist into the future but the cold environments of the Polar Regions are already changing. The Arctic is now experiencing some of the most rapid and severe climatic change on earth. Over the next 100 years, climate change, including changes in UV-B radiation and cloud cover, is expected to accelerate and to have major consequences for the Arctic and also the rest of the world. It is therefore timely to determine if adaptations of plants to extreme environments will constrain their responses to climatic warming. This presentation will show how adaptations to extreme environments will limit plant responses to climate warming, with implications for the biodiversity of extreme, cold environments. It will also give examples of pre-adaptation to high levels of UV-B radiation that will confer fitness if stratospheric ozone depletion continues in the Arctic. Plants of extreme environments change their environment. Some local environmental engineering by plants facilitates colonisation by plants of less extreme environments and a succession is established. Other feedbacks from plants to the environment potentially affect climate and light regime. Examples are reflectivity of surfaces, trace gas fluxes, and biogenic aerosols. This presentation will discuss some of these processes as a context for presentations within the symposium.

IL151

Tracking ancient UV-B fluxes

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The spores and pollen of terrestrial land plants increase their investment in UV-B screening pigments when exposed to elevated levels of UV-B radiation. Here, we report an increase in UV-B protecting pigments from a historical record of the spores of *Lycopodium magellanicum* growing in South Georgia and exposed to a progressive thinning of stratospheric ozone and a corresponding increase in UV-B radiation. Our data records a strong three fold linear increase in the concentration of UV-B protecting pigments in response to a 14% thinning of the ozone column. Our results were obtained using micro FT-IR analysis of sporopollenin, which is readily preserved in the fossil record. Therefore, this newly identified response offers a potential tool for

investigating natural changes in the stratospheric ozone layer and UV-B flux over geological time.

We are initially targeting the Permian - Triassic (P-Tr) mass extinctions (~250 million years before present) for further investigation. This is the largest and most prolonged of all the mass extinction events that have occurred over the last 500 million years. Recent work has identified the occurrence of lycopsid spores in permanent tetrads at the P-Tr boundary as evidence of mutagenesis driven by a global collapse in the stratospheric ozone layer and a corresponding increase in the flux of UV-B radiation at the earth's surface. Underlying agents put forward to explain this phenomenon are the eruption of the Siberian Traps volcanic complex and the release of hydrogen sulphide as a result of oceanic anoxia combined with a precipitous decline in atmospheric oxygen. This hypothesis can now be thoroughly tested using a combined approach, involving the examination of extant plants subjected to experimentally manipulated UV-B radiation, the geochemical study of fossil spores and pollen and the long-term modelling of stratospheric ozone.

IL152

Can marine microorganisms influence the melting of the Arctic pack ice?

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There is no place on earth where the climate change faster than it does in the Arctic with an observed warming about twice the global average during the past two decades causing enhanced melting of snow and sea-ice. The melting of the sea-ice will not only have dramatic consequences for society but also strongly influence the feedback mechanisms between the ocean, sea-ice, clouds and radiation and not least marine ecosystems. So, can marine microorganisms influence the melting of the Arctic pack ice?

The answer is contending with many unknown factors. For example, will biological activity and production of cloud condensation nuclei (CCN) necessary for cloud formation increase or decrease with melting of the pack ice, and will resultant changes in warmer oceans oppose or reinforce the Arctic changes?

One proposed biological influence on radiation suggests that dimethyl sulfide (DMS) produced by marine phytoplankton is oxidized in the atmosphere to sulfuric acid, aiding the nucleating of particles that grow to become CCN. Sulfate-containing aerosols are ubiquitous in the atmosphere and usually the most numerous particles capable of acting as CCN, so that the theory seems very reasonable. But does DMS alone control the number of CCN? Could there be other biological controls on CCN in remote marine air? To help answering these questions three Arctic Ocean summer Experiments to the area north of 80°N in 1991, 1996 and 2001 on the Swedish icebreaker *Oden* were launched.

The observations have presented us with a new picture of the evolution of the remote Arctic aerosol. DMS will determine the mass of sulfate produced but will have only a minor influence on the number of CCN and thus cloud droplets, which will be dictated by the number of airborne particles originating in the surface microlayer of the open leads. This invalidates the DMS hypothesis and poses a stronger possible link between marine biology, cloud properties and climate than is provided by DMS alone.

IL153

Cryophilic cyanobacteria and algae and their succession in deglaciated landscapes

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Retraction and melt down of land-based glaciers are one of the most convincing signs of global warming. Cyanobacteria and algae play

a key role in the ecology of polar regions as starters of primary production. In freshly deglaciated areas the microbial primary succession processes have been studied only sporadically. They include three stages: invasion, colonisation and establishment, and successional development (this stage itself can be subdivided from early to late successional phase). Cyanobacteria and algae fix nitrogen, sequester carbon, accumulate mineral nutrients and store energy in their cells. Other microorganisms and higher plants participating in the overall succession processes following deglaciation subsequently use these accumulated elements and stored energy. In a favourable microclimate the turnover of these processes can be very fast. In contrast, in unfavourable conditions the turnover is slow, taking place much longer. A review of the present state of knowledge dealing with the above issues and results of three original experiments, conducted in the Arctic and the Antarctic regions will be presented and discussed.

PL154

Looking for novel circadian clock mutants in *Arabidopsis*

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To optimise growth and development in a 24-h day/light cycles, organisms have developed an endogenous circadian clock. The circadian system allows organisms to anticipate and time biological processes to a portion of the cycle. For the circadian clock to be useful, however, the time measured by the clock (subjective time) must be synchronised with the objective time of the environment. Nature provides a complex set of signals, including variation in temperature, light quality and quantity, over the day/night cycle that enables plants to reset their endogenous clock reliably. Global transcriptome studies demonstrated that at least 6% of *Arabidopsis* genes are rhythmically expressed with expression peaks at all phases throughout the day and night. The known clock mechanisms of mammals, insects, fungi and plants seem to have evolved independently; at the molecular level, however, each of them includes a gene circuit with negative feedback. These clock genes maintain the molecular oscillation that drives all other rhythms and any pathway involved in entraining the clock must affect the expression of at least one of these clock components. The central oscillator in *Arabidopsis* involves three members of two small gene families. The mutual regulation of the three genes is described in a molecular model. The model captures a number of data sets but is incomplete and the molecular events mediating light-dependent entrainment of the central clock are largely unknown. To obtain additional information about these molecular mechanisms, we performed a large-scale genetic screen and isolated several novel mutants displaying impaired circadian phenotypes. Characterisation of these mutants at the physiological and molecular level is in progress. Data concerning contribution of these genes to maintain a functional circadian system in *Arabidopsis* will be discussed.

IL155

The geometry and spectral aspects of ocular exposure influences risks

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Studies of the effects of ultraviolet radiation and intense light upon the eye infrequently cite the apparent variation in susceptibility of different parts of the cornea, lens or retina in animal models. Furthermore, environmental studies of sunlight generally ignore the importance of the geometry of sunlight exposure. Few epidemiological studies pay appropriate attention to the variation of cataract with different geometrical factors in the environment, such as ground reflectance. The reduced exposure of the eye by the

upper lid, or the impact of the change in solar spectrum depending upon the position of the sun in the sky is seldom considered in epidemiological studies. When the sun is low in the sky and readily in the field-of-view, it appears yellow or orange—demonstrating the greatly reduced fraction of short-wavelength light and UV present in the spectral distribution. When the short-wavelength component of sunlight is most intense, the sun is overhead and direct exposure of ocular structures is very limited by the upper lid. Only when the ground surface is highly reflective, as when snow is on the ground, is the eye exposed to substantial levels of the particularly damaging short-wavelengths in sunlight. By carefully examining the geometrical distribution of age-related changes in the cornea, lens and retina, a stronger causal relation can be argued for the role of sunlight in some age-related ocular changes and pathologies in the eye.

IL156

Light damage to the retina: model and reality

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Light is essential for most life on earth but it can also threaten ocular health. Animal experiments demonstrate that light exposure at high doses induces cell death of photoreceptors and pigment epithelium. Cell death occurs by apoptosis, a gene -regulated demise which is a ubiquitous, basic phenomenon in biology and medicine. Apoptosis becomes increasingly important in cancer, immunology and neurodegeneration. Apoptosis was found to be the common death pathway in retinal degenerations in humans and animals. Bright light exposure enhances degeneration in several animal models and possibly some human retinal degenerations. To understand apoptotic mechanisms we developed models of light - induced apoptosis. Free moving rodents are exposed to white fluorescent light for 1 - 2 h followed by functional analysis or killed at different time points after exposure and subsequent morphological and molecular biological analysis. Similar analyses are applied after exposure of anesthetized animals to high energy blue light (410 +/- 10 nm; 430 +/- 10 nm) in the minute range. High level white light - induced apoptosis depends on the transcription factor AP1 (activator protein 1) and can be completely inhibited by blocking AP1 activity by dexamethasone. The rate of metabolic regeneration of the rod visual pigment rhodopsin is a crucial determinant for susceptibility to white light damage. By contrast, blue light - induced lesions are independent of AP1, cannot be blocked by dexamethasone and do not depend on rhodopsin regeneration rate. Apart from being a model system, light damage occurs in human and animal retina: solar retinopathy is well known to ophthalmologists. Whether light can enhance disease progression in age related macular degeneration (AMD) and retinitis pigmentosa (RP) is still a debated issue. Experimental evidence indicates that light can enhance aging of the retina. Thus, inhibition of apoptosis may open therapeutic strategies for retinal degenerations.

IL157

The photoreactivity of retinal melanin and lipofuscin granules

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Melanosomes are formed in the retinal pigment epithelium (RPE) during embryogenesis and undergo age-related changes while lipofuscin granules accumulate throughout life and are associated with age-related macular degeneration. Melanosomes appear to undergo photooxidative changes with increasing age resulting in an increase of oxygen uptake, and photogeneration of superoxide anions. Exposure of cultured RPE cells to both aged human melanin and blue light results in an abnormal morphology with cells absent from the monolayer. Overall cell viability is reduced by 60% at 48 hours as compared to human melanin-containing cells

maintained in the dark. By contrast, cells containing young melanosomes only demonstrate a 10% decrease in cell viability. Lipofuscin granules are potent photogenerators of reactive oxygen species; (i) exposure to blue light results in the photogeneration of triplet excited states, singlet oxygen, superoxide anions and hydroxyl radicals and lipid hydroperoxides, (ii) reactive species photogenerated by lipofuscin are sufficient to allow lipid membrane destabilisation and loss of enzyme activity, (iii) lipofuscin photoreactivity increases with age, (iv) exposure of lipofuscin-containing RPE cells to blue light results in membrane peroxidation, loss of lysosomal stability and cell death which is not observed in the dark or in cells exposed to green light and (v) nuclear DNA strand breaks correlate with the photoreactivity of lipofuscin. The photoreactivity of lipofuscin appears to be significantly greater than that of melanosomes on a per granule basis. In conclusion, the increased blue light-induced photoreactivity of melanosomes with age together with the accumulation of highly photoreactive lipofuscin granules provide a source of reactive oxygen species leading to depletion of vital cellular reductants and contribute to RPE cell dysfunction.

IL158

Optical radiation effects upon the lens

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Optical radiation includes radiation in the wavelength region from 1 nm to 1 mm. The crystalline lens is situated behind the cornea and the lens. The exposure of the lens therefore strongly depends on the attenuation of optical radiation in these ocular media and for linear attenuation on the absorption in the lens. Numerous artificial sources emit optical radiation. The most important source for humans is the sun. Given solar irradiance on the earth, there is no acute damage in the lens. However, several epidemiological studies have shown an association between cataract and long term chronic exposure to medium energy ultraviolet radiation type B (UVR-B) from the sun. Artificial sources of optical radiation can emit in any part of the spectrum and the effect depends on wavelength and exposure time. Low intensity UVR-B causes photochemical effects that lead to acute permanent cataract development during a week. Then, the cataract remains constant. UVR-B penetrates only half a millimeter. At the cellular level, UVR-B causes loss of cat-ion balance, DNA damage and p53 expression close to the anterior surface of the lens. There is a marked species variation in sensitivity. At constant dose, there is a maximum sensitivity in the time window 5-120 min.. If the exposure is fractionated in two equivalent exposures, there is a maximum toxicity with an inter-exposure interval of on the order of 48-72 hrs. There is epidemiological evidence that long term chronic exposure to infrared radiation (IRR) sources such as glowing metal or glass can cause cataract thermally. High intensity IRR lasers may also cause cataract thermally. Further, extremely high intensity, nanosecond or shorter laser pulses anywhere in the optical radiation spectrum, that are transmitted to the lens, may cause mechanical disruption in the lens that is manifested as cataract.

IL159

UVA modulation of iron and heme homeostasis – a crucial role for heme oxygenase

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Previous studies from this laboratory have shown that ultraviolet A (UVA) irradiation of human skin fibroblasts not only strongly induces heme oxygenase 1 which catabolises heme to release free

iron but also directly leads to an immediate increase in the labile iron pool as a result of ferritin degradation as well as free heme release. Both free heme and iron have been implicated in the exacerbation of inflammatory responses. Maintenance of access to these components for the synthesis of cellular proteins requires exquisite control to avoid potential cell and tissue damage. Heme oxygenase is not only involved in heme and iron homeostasis but also the traffic of iron through the appropriate compartments to ensure its availability at safe levels for cellular functions. Both oxidative (e.g. UVA) damage to cells and tissue as well as inflammatory responses appear to disturb these homeostatic mechanisms and lead to a rapid up-regulation of heme oxygenase 1 which in turn participates in the restoration of non-damaging levels of heme and iron and prevents further damage. Although lacking experimental verification, such a mechanistic pathway almost certainly underlies the strong anti-inflammatory activity of heme oxygenases. It therefore follows that manipulation of HO-1 regulatory pathways, including transcriptional activator proteins (e.g. Nrf2) or Bach proteins (for down-regulation) may provide a direct route to influence the protective and anti-inflammatory responses mediated by the enzyme. Heme is involved in regulation of both Nrf2 and Bach1 and recent data obtained by visualising these proteins and using specific constructs over-expressing negative regulatory proteins supports the involvement of both these proteins in UVA regulation of HO-1 activity in human skin fibroblasts.

IL160

Role of cell cycle checkpoint proteins in UVA-induced S-phase arrest in mammalian cells

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Human cells have evolved DNA damage checkpoint pathways to regulate cell cycle transitions and facilitate DNA repair processes. Key components of this DNA damage-initiated cell cycle checkpoints include the ataxia-telangiectasia-mutated (ATM) and ATM- and Rad3-related (ATR) protein kinases. Upon stress-induced DNA damage, ATM and ATR phosphorylate a plethora of target proteins (such as p53, Chk1, Chk2...) involved in the DNA damage checkpoint response leading to cell cycle arrest at G1/S, S and G2/M phases. If such response has been characterized for UVC-induced DNA damage, to date, little is known about the effect of UVA radiation on the DNA damage checkpoint activation especially at S phase of the cell cycle in mammalian cells.

In the present study, we show that UVA radiation trigger a dose dependent S-phase arrest that is not abolished in cells lacking ATM, expressing a low level of ATR or over-expressing a dominant negative form of ATR. Furthermore, DNA synthesis inhibition is not abolished when cells were incubated with caffeine, an inhibitor of ATM- and ATR-dependent signalling pathway. These results suggest that UVA-induced S-phase arrest, unlike UVC, is independent of ATM and ATR signalling pathways. This is further supported by the fact that in cells inactivated for Chk1 using small interfering RNA (siRNA) or in Chk2-deficient cells UVA radiation also induces S-phase arrest. On the other hand, lowering the production of UVA-induced ROS by pre-treatment of cells with the anti-oxidant N-acetyl-L-cystein partially reverses the S-phase arrest. Finally, we provide evidence that vitamins but not amino acids present in the medium play a major role in UVA-induced S-phase arrest. Collectively, our data suggests that this arrest cannot simply be attributed to the presence of UVA-induced DNA lesions such as CPDs or 8-oxoguanine but rather to yet unidentified DNA perturbation that remained to be characterized.

IL161**UV responses in NER-deficient mouse keratinocyte cultures**

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The epidermis, the outermost layer of our skin, consists mainly of keratinocytes and suffers most directly from environmental ultraviolet radiation. The human genetic disease xeroderma pigmentosum (XP) and to a lesser extent Cockayne syndrome (CS) or trichothiodystrophy (TTD) have revealed the devastating effects of defects in nucleotide excision repair (NER) in skin exposed to UV light. Although genetically modified mouse models have proven invaluable for studying the importance of NER at the level of the whole organism, there is also a need for more controllable and manipulatable *in vitro* models to support and extend *in vivo* findings. Therefore long-term keratinocyte cultures derived from the skin of wild-type and NER-deficient mouse mutants were established. The cell lines retained their normal ability to engage into terminal differentiation when triggered with high calcium concentrations or after suspension in semi-solid medium. *Xpd^{TTD}* keratinocytes were disturbed in their ability to terminally differentiate *in vitro*, in line with earlier *in vivo* results. Survival analysis and DNA damage responses confirmed the exquisite sensitivity of *Xpa*, *Xpc* and *Csb* keratinocytes to UV irradiation, but also clearly delineated underlying differences in response. After a dose of UV-B that did not affect wild-type keratinocytes, *Xpa* keratinocytes showed a transient increase in S-phase arrested cells, followed by massive apoptosis. *Csb* keratinocytes responded to the same UV-B dose by a more sustained increase in S-phase arrested cells and were clearly more resistant to UV-B induced apoptosis than their *Xpa* counterparts. Irradiated *Xpc* keratinocytes responded exclusively with an S-phase arrest, in complete absence of apoptosis. Apparently, tumor-suppressive DNA damage responses are greatly dictated by the specific NER deficiency, highlighting the complex relationship between genome care-taking systems, cell cycle regulation, apoptosis and cancer susceptibility.

IL162**Mono-ubiquitination of PCNA: a key modification for the polymerase switch after UV irradiation in human cells**

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DNA polymerase η carries out translesion synthesis (TLS) past UV photoproducts and is deficient in individuals with the variant form of xeroderma pigmentosum. Pol η is mostly localised uniformly in the nucleus, but is associated with replication foci during S phase. Following treatment of cells with UV-irradiation or chemical carcinogens, the number of cells in which pol η accumulates into foci increases dramatically. These foci are sites at which replication forks are stalled at DNA damage. The C-terminal 120 aa are needed for nuclear localisation and relocalisation into foci. This region contains a nuclear localisation signal, a putative C₂H₂ zinc finger and a PCNA binding domain, all of which are required for relocalisation of pol η into foci. Pol η truncations lacking the C-terminal 120 aa fail to correct the defects in XP-variant cells. However, we currently understand very little about the mechanisms for polymerase-polymerase interactions taking place at the replication fork when a replication complex encounters a lesion. Here, we report results which could explain how this “polymerase switch” occurs. Following UV treatment, PCNA is mono-ubiquitinated (mUBI-PCNA) in human cells. These PCNA modifications are independent of the NER pathway, of the presence of pol η or of BRCA1 but dependent of Rad18. We observed that pol η and mUBI-PCNA become bound to the chromatin after UV-

irradiation. Most of the chromatin-binding fraction of pol η corresponds to the intranuclear foci detected by immunofluorescence. Interestingly, we have demonstrated that pol η interacts physically with mUBI-PCNA but not with unmodified PCNA after UV irradiation suggesting that this interaction is the “key” which “unlocks” replication forks stalled at sites of UV damage, by allowing translesion synthesis to take place.

OC163**RhoB up-regulation induced by UVB controls survival response of human keratinocytes**

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Ultraviolet B irradiation is directly absorbed by DNA and protein, and as such generates many deleterious effects on skin tissue leading to fast adaptive cell response that contributes to maintaining their functions and survival. Dysregulation of this response promotes skin cancers and premature photo-aging. In addition to genetic alterations, inappropriate growth, differentiation, and/or apoptotic response to UV plays key roles in photocarcinogenic process. The protein RhoB is a short-lived small GTPase involved in vesicle trafficking, cytoskeletal organization, cell growth and survival. RhoB was proposed as an early responsive gene to growth factors and DNA damaging agents such as UV light and ionizing radiations.

In this study we have investigated the RhoB expression control and its cellular consequences after UVB irradiation in human keratinocytes. Our results show that RhoB is rapidly and extensively activated (increase in GTP-RhoB) after environmental relevant dose of UVB radiation in HaCaT keratinocytes and in primary cultured keratinocytes. This early activation is EGFR-independent and is followed by an EGFR-dependent increase of RhoB expression allowing the maintenance of high levels of GTP-RhoB. This protein induction is a result of the induction of RhoB promoter activity leading to an increase in RhoB mRNA sustained by its stabilization.

One of the major response of keratinocytes to UVB irradiation is apoptosis. Thus we have tested the role of RhoB in UVB-induced apoptosis. Specific inhibition of RhoB induction by RNAi increases the UVB apoptotic response on HaCaT cells (2-fold). Moreover, RhoB inhibition inhibits the UVB-induced Akt but not p38 MAPK phosphorylation. Consistent with this, over-expression of RhoB seems to increase HaCaT survival after UVB irradiation. Our results put on light RhoB as a protective determinant in UVB cellular response and suggest its potential role in photocarcinogenesis.

OC164**Overexpression of phospholipid hydroperoxide glutathione peroxidase in human dermal fibroblasts abrogates UVA irradiation-induced expression of interstitial collagenase/matrix-metalloproteinase-1 by suppression of phosphatidylcholine hydroperoxide-mediated NF κ B activation and interleukin-6 release**

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Phospholipid hydroperoxide glutathione peroxidase (PHGPx) exhibits high activity in reducing phosphatidylcholine

hydroperoxides (PCOOHs) and may play a central role in protecting the skin against UV irradiation-triggered detrimental long-term effects like cancer formation and premature skin aging. We addressed the role of PHGPx in the protection against UV irradiation-induced expression of matrix-metalloproteinase-1 (MMP-1). We created dermal fibroblast cell lines overexpressing human PHGPx. In contrast to a maximal 4.5-fold induction of specific MMP-1 mRNA levels in vector-transfected cells at 24 h after UVA irradiation, no MMP-1 induction occurred after UVA treatment of PHGPx overexpressing fibroblasts. As interleukin-6 (IL-6) was shown to mediate the UVA induction of MMP-1, we studied whether PHGPx overexpression might interfere with the NF κ B-mediated IL-6 induction and downstream signaling. Using transient transfections of IL-6 promoter constructs containing NF κ B binding sites, we observed a high induction of the reporter gene luciferase in vector-transfected control cells and a significantly lower induction in PHGPx-overexpressing fibroblasts following UVA irradiation. Consistently both UVA irradiation and treatment of fibroblasts with PCOOHs led to phosphorylation and nuclear translocation of the p65 subunit, whereas cells overexpressing PHGPx exhibited impaired NF κ B activation, p65 phosphorylation and nuclear translocation. In line with this, the PHGPx-overexpressing fibroblasts showed a reduced constitutive and UVA irradiation-induced IL-6 release. After incubating PHGPx-overexpressing cells with PCOOHs a reduced induction of IL-6 was observed. This together with the suppression of UVA irradiation-induced IL-6 release in the presence of Trolox, a chain breaker of PCOOH-initiated lipid peroxidation, indicates that UVA irradiation-induced PCOOHs and lipid peroxides initiate the NF κ B-mediated induction of IL-6, which mediates the induction of MMP-1.

IL165

Analysis of state transitions in *Chlamydomonas* and *Arabidopsis*
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State transitions involve a rebalancing of the light absorption capacity of the antennae of photosystem 2 (PSII) and PSI upon unequal excitation of these two photosystems. Overexcitation of PSII relative to PSI leads to the reduction of the plastoquinone pool and to the activation of a protein kinase through the cytochrome b_6/f complex and to the phosphorylation of LHCII which is displaced subsequently to PSI (state 2). The process is reversible as the kinase is inactivated upon oxidation of the plastoquinone pool and LHCII is dephosphorylated and moves back to PSII (state 1). In *Chlamydomonas* transition from state 1 to state 2 also occurs in response to a drop in cellular ATP levels. In this alga this transition corresponds to a switch from linear to cyclic electron flow. We have used a genetic approach in *Chlamydomonas* and isolated several mutants deficient in state transition. Most of these mutants are blocked in state 1 and deficient in LHCII phosphorylation. One of the mutants, *stt7*, has been found to be deficient in a Ser-Thr protein kinase that is associated with the thylakoid membrane. An ortholog of the *Stt7* kinase, called *Stn8*, exists in *Arabidopsis*. Using a TDNA insertion line with disrupted *Stn7* we have shown that the *Stt7* and *Stn7* kinase are not only structurally but also functionally related because the *stn8* mutant is deficient in state transition and unable to phosphorylate LHCII. We are using site-directed mutagenesis to elucidate the role of the different domains of *Stt7* and *Stn7* and the yeast two hybrid screen for identifying novel interactants with these two kinases. We are also characterizing several new state transition mutants some of which grow poorly under phototrophic growth conditions.

IL166

Elucidating the Mg-ProtoIX mediated signalling pathway

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The expression of nuclear genes encoding plastid proteins is regulated by multiple signals that originate in the plastids. These plastid signals are critical during the development of chloroplasts from plastids, initiating the expression of nuclear photosynthetic genes. Furthermore, plastid signals communicate changes in the status of chloroplasts during stresses such as low temperature and high light. To date, at least three different signalling pathways have been identified that originate in the plastid and control nuclear gene expression. We identified the tetrapyrrole Mg-ProtochlorophyllinIX (Mg-ProtoIX) as a signalling metabolite communicating the state of the chloroplast to the nucleus (Strand et al., 2003). Mg-ProtoIX accumulates in the plant cell during stress and a large number of nuclear encoded photosynthetic genes are regulated by the accumulation of Mg-ProtoIX.

The remaining question is how the Mg-ProtoIX signal is transduced from the chloroplast to the nucleus. Firstly, is Mg-ProtoIX exported from the chloroplast where it is synthesized to the cytosol? To address this question it was possible to take advantage of the photoreactive property of Mg-ProtoIX. Mg-ProtoIX can be visualized using a confocal laser-scanning microscope. Our images demonstrate that Mg-ProtoIX is exported from the chloroplast and accumulates in the cytosol. Second, what cytosolic components recognize the accumulation of Mg-ProtoIX? To reveal cytosolic and nuclear components involved in the Mg-ProtoIX-mediated pathway, we have pursued proteomic and genetic approaches. We have identified several interesting Mg-ProtoIX binding proteins and identified a suppressor mutant, *sog1* for one of the genome uncoupled mutants (*gun5*) affected in the Mg-ProtoIX pathway. A working model of the Mg-ProtoIX mediated signalling pathway will be presented based on our results.

IL167

Carbon metabolite sensing and signalling: the role of the trehalose pathway

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Sucrose and trehalose are the most widespread non-reducing disaccharides in nature. Sucrose functions as transported energy and carbon source and protective molecule in plants, and trehalose has these functions in fungi, bacteria and insects. Until recently, with very few exceptions, this division of sucrose and trehalose between the kingdoms was thought to be clear cut. However, it now appears that the trehalose pathway has not only been retained in plants over evolution, but has diversified to play a central role in the newly emerging carbon metabolite sensing and signalling systems. In *Arabidopsis* there are more genes encoding enzymes of trehalose synthesis than of sucrose synthesis. The trehalose pathway and trehalose 6-phosphate, in particular, have been shown to play an indispensable role in plants influencing processes like photosynthesis, sugar metabolism, disease and stress resistance. This talk will address the role of the trehalose pathway with particular focus on the interaction with sucrose metabolism and the regulation of photosynthesis.

IL168

Chloroplast-mediated regulation of *Arabidopsis* transcriptome

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We have studied nuclear transcript accumulation in fully expanded *Arabidopsis* leaves with respect to light-induced chloroplast signals. *Arabidopsis* plants were exposed to different quantities of

white light, lights favouring PSII excitation or PSI excitation and to darkness. After 3 hours, the excitation pressure of PSII, state transitions and the steady state phosphorylation of thylakoid proteins, net CO₂ fixation and the stromal redox state were recorded, and the mRNAs were extracted from leaves and subjected to 8K cDNA microarrays. 2027 genes changed their expression at least in one light treatment, as compared to the control growth light condition. 370 of them were targeted to chloroplasts and 150 to mitochondria.

Only a few genes were regulated by the redox state of the plastoquinone pool in our short-term illumination experiments. More genes responded directly to the net CO₂ fixation rate of the leaves, which varied depending on both the quantity and quality of light. This group included e.g. the genes for trehalose phosphate synthase, GAST1, isocitrate lyase and DnaJ. Stromal redox state, measured as an activity of NADP-MDH and as an inhibition of LHCII protein phosphorylation, seemed decisive for the expression of specific chloroplast genes, including representatives of the *lll* and *ftsH* families. Genes encoding the oxygen evolving complex proteins of PSII were specifically upregulated at low fluence rates independently of the quality of light, whereas the expression of the *lhca* and *lhcb* genes was more differentially regulated.

OC169

Singlet oxygen as a stress factor and signal in *Rhodobacter*

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Facultative phototrophic prokaryotes like *Rhodobacter* encounter photooxidative stress caused singlet oxygen when pigmented cells are exposed to light and oxygen. The effect of singlet oxygen produced by BChl *a* *in vivo* and by the photosensitizer methylene blue were investigated under aerobic growth conditions in *R. sphaeroides*. Carotenoids are pivotal to defend stress caused by singlet oxygen, but relative carotenoid concentrations and expression of carotenoid biosynthesis genes were not increased by singlet oxygen. Since an adaptation to singlet oxygen induced stress was observed in *R. sphaeroides* other defense systems have to be present. To identify such defense systems in cultures exposed to singlet oxygen proteins were labeled *in vivo* by the addition of ³⁵S-methionine and analyzed by 2D-gel electrophoresis. The synthesis of more than 15 soluble proteins was strongly increased and a total of 60 proteins was changed in synthesis rate. The identified proteins are involved in the degradation of peroxides, have hydrolytic or proteolytic function, are subunits of peptide transport complexes and are involved in glutathione metabolism. The addition of hydrogen peroxide and paraquat gave rise to distinct protein patterns suggesting that the response to singlet oxygen was specific. However, the synthesis of some proteins was induced by all three reactive oxygen species indicating their involvement in general oxidative stress response. The expression of proteins was verified for selected genes by determining relative mRNA levels using quantitative real-time RT-PCR. Upstream regions of genes induced by singlet oxygen contained putative binding sequences for RpoE, an σ^E -factor. In an *rpoE* deletion mutant some proteins induced by photooxidative stress are missing, confirming a transcriptional control of genes involved in the defense against photooxidative stress in *R. sphaeroides*.

OC170

Molecular events underlying excitation energy control in the Photosystem II antenna

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For the maximum efficiency in the use of light energy, plants have light-harvesting antennae. It collects photons and delivers their energy to the photosynthetic reaction centres. Antenna is designed

to respond to the extreme changes in the intensity of sunlight encountered in nature. In shade, it efficiently harvests light with minimum losses of its energy. However, in full sunlight, much of the energy absorbed is not used. At these conditions the mechanism exists to switch antenna to a specific state, which safely dissipates the excess energy as heat. This state was found to be essential for plant survival because it provides protection against the potential photo-damage of the photosynthetic machinery and the thylakoid membrane itself. Whilst the features that establish high photosynthetic efficiency have been identified, almost nothing is known about the molecular nature of dissipative states. Based on a number of biochemical and spectroscopic approaches performed *in vitro* on the LHCII antenna complexes, which effectively dissipate excitation energy into heat (protein aggregates and LHCII crystals) we came to the conclusion that the available structure of this complex represent the efficiently dissipating state. We conclude that LHCII antenna has indeed an intrinsic capacity to regulate the energy flow and discuss the possible molecular features involved. The role of PsbS, an LHC-related protein, which is essential in formation of the photoprotective energy dissipation, is also considered as well as the involvement of the xanthophyll cycle carotenoids. All the data obtained are consistent with the allosteric model of light-harvesting control, where the xanthophyll cycle plays modulating role, PsbS is a key proton receptor and signal transducer into the antenna, switching it between efficient and inefficient, photoprotective modes.

IL171

Regulation of DNA repair in E2F1 transgenic and knockout mice

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The transcription factor E2F1 suppresses tumor development. We have found a strong correlation between expression of the transcription factor E2F1 and the efficiency of nucleotide excision repair (NER) in knockout and transgenic mice. If this is correct, then a reduction in DNA repair efficiency may account for the cancer predisposition of *E2f1*^{-/-} mice. The mechanism by which E2F1 stimulates NER is unclear. One possibility is that E2F1 regulates the expression of one or more rate-limiting factors involved in NER. Another more intriguing possibility is that E2F1 directly interacts with DNA damage thereby increasing the efficiency of DNA repair during its early stages. The presence of E2F1 at or near a damaged site could facilitate binding of damage recognition proteins to the DNA damage or to the E2F1 protein itself. In this scenario, E2F1 would behave like XPC-hHR23B, increasing the efficiency of damage recognition. Alternatively, E2F1 could destabilize chromatin at the site of damage and induce a configuration more accessible to damage recognition. We have shown that E2F1 binds DNA damage induced by UV. Support for the "molecular hijacking" of E2F1 and possible role in damage recognition comes from co-localization experiments in which E2F1 and specific photoproducts are found in the same regions of cells UV-irradiated through polycarbonate filters. In other preliminary studies we used electromobility shift assays to show that binding of purified E2F1 to its consensus sequence is mitigated in the presence of a pyrimidine homopolymer irradiated with UVC. Increasing the amount of UV-polymer or UV dose was equally effective in inhibiting binding. Taken together these data support the hypothesis that E2F1 and, perhaps, other transcription factors may function as surrogate DNA damage recognition proteins and, as such, modulate the efficiency of NER in mammalian systems.

IL172**DNA lesions and mutations induced by ultraviolet A and B radiation in human and mouse cells***G.P. Pfeifer, A. Besaratinia**Department of Biology, Beckman Research Institute of the City of Hope, 1450 East Duarte Road, Duarte, CA 91010, USA*

The ultraviolet components of sunlight (UVA and UVB) are implicated in the etiology of human skin cancer. The underlying mechanism of action for UVB carcinogenicity is well defined, however, the mechanistic involvement of UVA in carcinogenesis is not fully delineated. We investigated the genotoxicity of UVA vs UVB in the overall genome and in the *TP53* tumor suppressor gene in normal human skin fibroblasts and in mouse embryo fibroblasts. In BigBlue® mouse fibroblasts UVB induced predominantly C to T transitions at dipyrimidine sites whereas UVA characteristically induced G to T transversions, which were enhanced by intracellular induction of photosensitizers. Immuno-dot-blot analysis identified the *cis-syn* cyclobutane pyrimidine-dimer (CPD) as a distinctive UVB-induced lesion, and confirmed its low-level formation in the genomic DNA of UVA-irradiated cells dependent on radiation dose. Also, a dose-dependent formation of pyrimidine (6-4) pyrimidone photoproducts was observed in the genomic DNA of UVB-irradiated cells only. High pressure liquid chromatography/tandem mass spectrometry analysis showed an induction of 8-oxo-7,8-dihydro-2'-deoxyguanosine in the genomic DNA of UVA- but not UVB-irradiated cells dependent on radiation dose. Mapping of DNA damages by terminal transferase-dependent polymerase chain reaction (TD-PCR) revealed site-specific but non-identical formation of polymerase-blocking lesions and/or strand breaks along exons 5-8 of the *TP53* gene in UVB- and UVA-irradiated cells. The UVB-induced lesions detected by TD-PCR were almost exclusively mapped to pyrimidine-rich sequences; however, the UVA-induced lesions were mapped to purine and pyrimidine-containing sequences along the *TP53* gene. Cleavage assays with lesion-specific DNA repair enzymes coupled to ligation-mediated (LM-PCR) showed site-specific but not identical formation of CPDs along the *TP53* gene in UVB- and UVA-irradiated cells. The hotspots and dose-dependency of CPD formation were much more pronounced in the UVB-irradiated cells relative to UVA-irradiated cells. Additionally, dose-dependent formation of oxidized and ring-opened purines, and abasic sites was established in the *TP53* gene in UVA- irradiated cells only. We conclude that UVA induces promutagenic CPDs and oxidative DNA damages at both genomic and nucleotide resolution level in normal human skin fibroblasts. These oxidative DNA damages, specially oxidized purines, are much more abundant than CPDs, which may explain the induction of G to T transversion mutations previously observed in UVA mutagenesis experiments.

IL173**Induction of CPD retaining basal cells after solar simulating UV-irradiation of human skin***B. Volkmer¹, S. Henning¹, D.L. Mitchell², E.W. Breitbart¹, R. Greiner¹*¹*Dermatologisches Zentrum Buxtehude, Buxtehude, Germany;*²*M.D. Anderson Cancer Center, University of Texas, USA*

We recently published that chronic UVB-irradiation leaves Cyclobutane-pyrimidine-dimer (CPD) retaining cells (CRBCs) in the epidermis of mouse skin which can be detected using a fluorescence-labelled monoclonal antibody against CPD. CRBCs were also found in human skin biopsies predominantly in samples excised from UV-exposed body sites.

In this study we further investigated the induction mechanisms of CRBCs in the basal layer of human skin. Healthy volunteers were irradiated with a chronic (0.3 MED daily for 5 days) and acute (1.5 MED) dose of solar simulating UV-irradiation (Solar-UV-simulator, L.O.T. Oriol). Immediately after the irradiation and 2 or 6 weeks later skin biopsies were taken, skin sections were prepared and stained with CPD antibody. Though we found a great interindividual variability according to repair of UV-damage,

CRBCs were detected in the majority of the samples up to 6 weeks after chronic as well as after acute irradiation.

With this experiments we could show, that CRBCs can be induced in human skin not only by an acute erythematous UV-dose but also by a chronic irradiation protocol using suberythematous doses. CRBCs can be observed up to 6 weeks after irradiation thus outlasting a regeneration cycle of the skin (about 4 weeks) which indicates an even longer persistence. Both attributes, the retaining of DNA damage and the persistence in the epidermis indicate that CRBCs could represent epidermal stem cells. Since epidermal stem cells are ideal targets for the carcinogenic effect of UV-irradiation, CRBCs could characterize an early step in skin cancer induction. Furthermore, the interindividual variability of the number of stem cells could define the individual skin cancer risk (the higher the number of stem cells the bigger the sensitive target). If it turns out that the CRBCs are precursors of (non-melanoma) skin cancer cells and correlate with the individual number of epidermal stem cells, they would be very important for the development of a molecular epidemiology of skin cancer and as a measure for an individual skin cancer risk.

IL174**Oncogenes, signal transduction and melanoma in *Xiphophorus****S. Meierjohann¹, C. Wellbrock², A. Gómez³, E. Geissinger⁴, C. Froschauer¹, E. Wende¹, M. Papp¹, A. Kraiss¹, M. Scharit¹*¹*Physiological Chemistry I, Biocenter, University of Wuerzburg, Germany;*²*The Institute of Cancer Research, London, UK;*³*Instituto de Acuicultura de Torre la Sal (CSIC), Spain;* ⁴*Institute of Pathology, University of Wuerzburg, Germany*

The *Xiphophorus* melanoma is an animal model for the function of receptor tyrosine kinases (RTK) in tumor formation. The neoplastic transformation of pigment cells as well as the succeeding steps of tumor evolution are brought about by the overexpression of a mutationally altered version of the EGF-receptor, designated *Xmrk*. *Xmrk* arose from the *EGFR* proto-oncogene by a gene duplication event, which changed the transcriptional control in a way that the oncogene is specifically overexpressed in pigment cells of certain hybrid genotypes. Two mutations in the extracellular domain lead to unpaired cysteines, which in the wildtype receptor are engaged in intramolecular cystin bridges. In the oncogenic receptor they form intermolecular bonds, thus generating constitutively active receptor dimers. *Xmrk* activates several signal transduction pathways including the ras/raf/MEK/MAP-kinase cascade, the PI3-kinase/PKB pathway, signaling through fyn and STAT5. These events lead to activation of proliferation, inhibition of differentiation, and anti-apoptosis in the pigment cells. *Xmrk* dependent activation of osteopontin expression enables the transformed pigment cells to survive in the dermal compartment. Through interaction with the focal adhesion kinase *Xmrk* induces migration. The malignant phenotype of the pigment cell tumors is controlled by several genetic factors, which influence the location and onset of primary tumor formation, the transition from radial to vertical growth phase, invasiveness, and the speed of tumor growth. Such phenotypic changes are accompanied by differences in protein levels and activation status of *Xmrk* target protein.

IL175**Role of cell cycle regulating genes in susceptibility to UV-induced melanomas in *Xiphophorus* hybrids***R.S. Nairn, R. Beard, D. Trono, A.P. Butler**The University of Texas M.D. Anderson Cancer Center, Science Park-Research Division, Smithville, TX USA 78610*

Xiphophorus interspecies hybrids offer genetic models of neural crest tumors, including UV-induced melanomas. First-generation backcross (BC₁) hybrids derived from backcrossing F₁ hybrids of *X. maculatus* strain Jp 163 B and *X. helleri* to the *X. helleri* parental strain exhibit segregation of susceptibility to UV-induced melanomagenesis. The *Xiphophorus CDKN2AB* gene has sequence homology to the mammalian CDKN2 family of cyclin-dependent

kinase inhibitors, and genetic linkage studies implicate it as a candidate tumor susceptibility gene in this model. Although human *CDKN2A* (p16) is frequently deleted, mutated, or silenced by methylation in human tumors, *Xiphophorus CDKN2AB* is overexpressed in UV-induced melanomas. We analyzed the activities of reporter plasmids containing either *X. helleri* or *X. maculatus CDKN2AB* promoters in *Xiphophorus* cell lines PSM (melanoma) and A2 (non-melanoma). Deletion analysis and site directed mutagenesis (SDM) of the *CDKN2AB* promoters identified several regions of potential regulatory importance. One is a putative Sp transcription factor binding site at +57 of the *X. helleri* promoter. *In vitro* binding studies indicate that the *X. helleri* promoter binds a factor related to Sp3 in nuclear extracts from *Xiphophorus* cells. Sp1 binding was not observed in these extracts, and the non-consensus site found in *X. maculatus* bound Sp3 only poorly. SDM suggests that Sp3 is a negative regulator of the *CDKN2AB* promoter in melanoma cells. Another region in the *X. helleri* promoter contains a partial duplication of the corresponding site in the *X. maculatus* promoter, with two copies of a motif resembling a conserved element (CR1) found in mammalian tyrosinase genes. SDM of either CR1 element dramatically reduces promoter activity in PSM melanoma cells, but has little effect on expression in A2 cells. Our results suggest a model in which overexpression of *CDKN2AB* in fish melanomas may be explained, in part, by enhanced promoter activity in melanoma cells.

OC176

Deficient UV induction of melanoma in HGF/SF transgenic recessive yellow (Mc1r^{e/e}) mice

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The HGF/SF transgenic mouse model for melanoma produces, in response to neonatal UV irradiation, cutaneous melanomas with histopathology and genetics which recapitulate human disease. We have used this model to investigate the role of the melanocortin 1 receptor (Mc1r) in melanoma. In humans, variants in the MC1R are associated with red hair and also with increased susceptibility to melanoma. The recessive yellow mouse C57BL/6J-Mc1r^e has a spontaneous mutation resulting in a truncated, non-functional Mc1r and has yellow hair but black eye pigmentation. The HGF/SF transgenic mouse on a C57BL/6 genetic background was crossed with C57BL/6J-Mc1r^e. Black transgenic animals, as previously reported, were readily identified from wild-type black littermates by the high levels and altered distribution of pigmentation, but yellow transgenic animals were not observably different from their yellow wild-type littermates. Visualization of melanocytes in adult yellow transgenic skin using antibodies to S100, Trp1 or Trp2 in contrast to black or FVB transgenics, did not reveal the presence of significant numbers of extrafollicular melanocytes. Black and yellow transgenic and wild-type littermates were UV irradiated with an F40 sunlamp emitting UVB, UVA and visible radiation at 3 days of age using an established protocol for initiating melanoma in this model. Transgenic black animals readily produced pigmented melanomas. In contrast, only rare black dermal melanocytic foci were observed in yellow transgenics. There were no melanomas in UV irradiated wild-type black or yellow animals or in unirradiated animals of each genotype. These findings indicate that the presence of pheomelanin per se is insufficient to promote melanomagenesis and suggest a hypothesis that aberrant rather than absent signaling through Mc1r may be responsible for susceptibility to melanoma in carriers of MC1R variants.

IL177

Photodynamic therapy-based inactivation of viruses in the presence of red blood cells

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Photodynamic therapy (PDT) is well known method of anticancer treatment. In last decade this therapy showed promises in inactivation of number of microbia as it was confirmed in numerous studies. Sterilization of blood and its products is challenging because red blood cells must function after this procedure. There is number of papers dealing with successful destroy of viruses in different media after PDT. Unfortunately, after PDT once photosensitizer is used, it remains in solution of e.g. red blood cells (RBC) or the whole blood, thus giving a risk of photosensitization of patient for many weeks when the RBC is going to be used again. Main aim of our project was to find photosensitizer for both enveloped and naked viruses, which does not cause damage to RBC nor it is present in solution after PDT. We have tested several model viruses: BVDV (bovine viral diarrhea), HSV-1 (herpes simplex virus-1), VSV (vesicular stomatitis) as models of enveloped viruses and EMCV (encephalomyocarditis) as representative of naked viruses. We have used a large number of photosensitizers: non-polar, amphiphilic and polar and later immobilized compounds anchored to solid matrices. The immobilized sensitizers wemesoketol, IVAN-11 and others. Our studies revealed that PDT with immobilized sensitizers shows promises in sterilization of blood however much effort is still needed to optimize the conditions of its application.

IL178

Antimicrobial PDT with phenothiazinium dyes: new mechanistic findings

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Phenothiazinium dyes such as methylene blue and toluidine blue O have long been proposed as antimicrobial photosensitizers (PS). They have the advantages of being readily available and are considered safe for human use. Their possession of a constitutive cationic molecular charge enables them to bind and penetrate both Gram-positive and Gram-negative bacteria as well as fungi. Nevertheless phenothiazinium dyes are comparatively less effective in mediating photoinactivation (PDI) of bacteria than other cationic PS with poorer visible absorption bands in the red wavelengths. We found that the efficiency of PDI with phenothiazinium dyes strongly depended on the cell density in the suspension as well as the dye concentration and whether dye was washed out from the cell suspension, for all three classes of microbes. Many antibiotics and toxic molecules are actively pumped out of bacteria by a range of multi-drug resistance pumps. By using knock-out and overexpressing mutants we have established that phenothiazinium dyes are substrates of the major facility superfamily pump NorA of *Staphylococcus aureus*, and of the resistance-nodulation-division family members MexAB-OprM of *Pseudomonas aeruginosa*, and also of TolC-AcrAB of *Escherichia coli*. Natural and synthetic inhibitors of these efflux pumps significantly potentiate the PDI of these bacteria by phenothiazinium dyes. *Bacillus* spores have previously been reported to be resistant to PDI. We have discovered that only phenothiazinium dyes out of a range of potential antimicrobial PS tested are able to mediate the destruction of *B. cereus*, *B. thuringiensis* (and to a lesser extent *B. subtilis* and *B. atrophaeus*) spores. In this case dye uptake by the spores appears to be mediated by passive diffusion.

IL179**Photodynamic therapy of bacterial and fungal infections with phthalocyanines: basic and pre-clinical studies**G. Jori¹, G. Roncucci²¹Department of Biology, University of Padova, Italy; ²Molteni Farmaceutici, Firenze, Italy

Phthalocyanines (Pcs) of cationic nature have been shown to act as efficient antimicrobial photosensitizers. These compounds have the advantage of allowing a thorough investigation as regards structure-activity relationships since their chemical structure can be modulated at several levels, including the peripheral substituents, the centrally coordinated metal ion and axial ligands to the central ion. Moreover, cationic Pcs display a high phototoxicity against a broad spectrum of pathogens, such as Gram(+) and Gram(-) bacteria, fungi, mycoplasmas and parasites; their photosensitizing activity is independent of the antibiotic-resistance spectrum of the microbial cell, hence even hardly curable pathogens, such as MRSA, can be rapidly and irreversibly inactivated. The cytoplasmic membrane is the main site of Pc binding and the initial target of the photoprocess, which prevents the selection of photoresistant strains, while the time-course and efficacy of the photoprocess is not affected by previous treatment with antibiotics. The safety of the treatment of microbial infections by PDT with Pcs has been successfully tested in animal models, as well as in dogs affected by spontaneously developed dermatoses, that had become chronic after repeated administration of antibiotics. On the basis of positive results obtained in toxicological studies, one Pc has been selected for phase I/II clinical trials focused on the topical treatment of specific cutaneous and oral cavity infections. This technique can be usefully applied also for the decontamination of microbially polluted waters.

IL180**Antimicrobial PDT: we have heard the theory, what about the practice?**

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The potential of PDT to destroy microorganisms is well-known with many sensitizers showing good activity *in vitro*. Bacteria, viruses, fungi and parasitic organisms have all been shown to be susceptible to PDT under appropriate conditions. There has been particular interest in antibacterial PDT because of the search for alternative approaches to conventional antibiotics, especially in combating resistant strains. In order to achieve successful clinical antimicrobial PDT, it is essential to be able to achieve good selectivity for bacterial cell kill ie with little or no host tissue damage. However, except for a very few single case studies, until now there has been no application of antibacterial PDT in patients.

We have developed a series of cationic photosensitizers in the phenothiazinium cation series, which have remarkably increased activity over known compounds such as methylene blue. After appropriate pre-clinical testing and the approval of ethical and regulatory authorities, one of these photosensitizers (PPA 904) has been formulated for topical use and applied in a trial in 10 patients with bacterially contaminated diabetic or venous ulcers. In all evaluable patients (eight), there was a substantial and significant decrease in bacterial load after treatment, compared with the bacterial load before treatment. Moreover, the antibacterial PDT was found to be safe and well-tolerated with no pain (unlike ALA-PDT). The treatment was complete in less than 30 minutes.

This first clinical study in antimicrobial PDT suggests that it has the potential to be a safe and effective approach.

OC181**Photosensitization-based inactivation of harmful mycomycetes**Ž. Lukšienė¹, D. Pečiulytė², A. Lugauskas²¹Institute of Materials Science and Applied Research, Saulėtekio 9, Vilnius 2040, Lithuania; ²Institute of Botany, Zaliuju ezeru 49, Vilnius 2021, Lithuania

Photosensitization technology is based on the interaction of two absolutely non-toxic agents – photoactive compound (photosensitizer) and visible light. Due to the fact, that photosensitizer is accumulated in microorganisms, the interaction of photosensitizer with light in the presence of oxygen produce radical-based cytotoxic reactions localized inside the cell which further cause total destruction of this microorganism.

Due to the existing disadvantages of methods applied to inactivate microorganisms fight against harmful and pathogenic microbes is still continuing. Thus, it is obvious, that novel, effective and environmentally friendly methods must be developed.

According to the data obtained, photosensitization using hematoporphyrin dimethyl ether (HPde) as photosensitizer and visible light (380< λ <680 nm) is able to inactivate *Trichotecium roseum*, *Fusarium avenaceum*, *Rhizopus oryzae* and *Aspergillus flavus*, *Alternaria alternata*, *Acremonium strictum*. The rate of inhibition is in clear correlation with the accumulated by microbe amount of HPde. At higher HPde concentrations (7×10^{-4} M) higher inactivation of micromycetes was detected. Of importance to note, that some specificity of this treatment was observed: the highest inactivation was detected in the case of *Aspergillus*, much lower – in the case of *Trichotecium*.

The other interesting approach might be the detection of microorganisms by fluorescence of HPde what is accumulated inside. Typical red fluorescence of HPde may be reliable marker to find alive microorganisms, to detect loci of their damage.

Summarizing, photosensitization as novel biophotonic technology might be used to inactivate harmful and pathogenic micromycetes, to sterilize or decontaminate from various surfaces in environmental friendly way. Moreover, clear, easy and cost-effective detection of viable microorganisms from fluorescence of accumulated photosensitizer may be useful in different fields of antimicrobial fight.

OC182**Porphyrin polyamine conjugates : a new strategy for antimicrobial chemotherapy**

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PDT is based upon the selective accumulation of photoreactive compounds (photosensitizers) in tumor tissue and on the production of singlet oxygen by irradiation of the sensitizer-enriched tumor with visible light which causes cell death and often complete tumor.

Recently, a nononcological application of PDT has been established. Indeed, photosensitization can represent a useful approach for the killing of microbial cells since it has been observed that several porphyrins and related compounds show phototoxicity against bacteria, fungi and yeasts, and can be used for PACT (Photodynamic Antimicrobial Chemotherapy). On the other hand, the appearance of multiresistant bacteria is a growing concern illustrated, for example, by the difficulty in getting rid of nosocomial infections; so, there is an urgent need to discover new antibacterial agents pertaining to structural classes distinct from currently used antibiotics.

In connection with our research program on porphyrins, we report the synthesis and the antimicrobial activity of porphyrins bearing polyamine units (spermine and spermidine).

UV-visible, MS (Maldi), ¹H (400MHz) and ¹³C (100MHz) NMR spectra of these compounds showed the expected signals.

Antimicrobial activity of the synthesized compounds were evaluated against *Escherichia coli* and *Staphylococcus aureus*.

OC183

Photodynamic killing of *Leishmania major* with cationic dyes

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Cutaneous leishmaniasis (CL) is a vector-borne disease caused by flagellated protozoa of the genus *Leishmania*. The current chemotherapy for CL is toxic and drug resistance is encountered. The development of a simple, effective, and low-cost treatment that can be administered in the field environment is needed to combat this disease. The feasibility of using photodynamic therapy (PDT) in the management of CL has recently been demonstrated. Because the efficacy of currently available PDT agents is low, we initiated a screening program designed to identify a photosensitizer (PS) that would show the maximal phototoxicity towards *Leishmania*. Our studies revealed that 5-ethylamino-9-diethylaminobenzo[*a*]phenoselenazinium chloride (EtNBSe) exhibits the maximal therapeutic effect (83.8 ± 0.3 % of killing at fluence rate 10 J/cm^2) at a minimal concentration ($15 \text{ nmol per } 2 \times 10^7 \text{ L. major promastigotes}$) in comparison with protoporphyrin IX (ppIX), δ -aminolevulinic acid, benzoporphyrin derivative (BpD) and 5-ethylamino-9-diethylaminobenzo[*a*]phenothiazinium chloride (EtNBS). Although 80-90% of parasite cells were destroyed by all PSs at 50 J/cm^2 , we found that as few as 1.06×10^9 molecules of EtNBSe were required for killing one parasite, compared with 5.70×10^9 molecules of EtNBS, 4.38×10^{12} molecules of BpD, and 1.67×10^{13} molecules of ppIX. Characteristic features of the intracellular distribution of the dyes were the fast diffusion of benzophenoxazine analogues through the lipid membrane and preferable cytoplasmic accumulation. The significant rate of destroyed *Leishmania* parasites (73.1%) immediately after EtNBSe-PDT (10 J/cm^2) implies that the basic mechanism of parasite destruction is likely due to the damage of the cellular membrane. High phototoxic activity of EtNBSe can also be achieved with other skin-tropic strains of *Leishmania* (*L. major* strain V1, LV39, 5-ASHK and *L. tropica* strain K27). Negatively charged *Leishmania* parasites seem to be highly attracted to cationic dyes such as benzophenoxazine analogues, with the highest intracellular uptake after 1 h incubation ($1.85 \times 10^{15} \text{ mol/cell}$ of EtNBSe) in comparison with the other studied PSs ($p < 0.05$). *In vivo* research should provide additional findings that cationic dyes can be very useful as photosensitizing agents in PDT against CL.

IL184

Ultrafast electron transfer in photosynthetic reaction centres: on the way to a molecular understanding of photosynthetic electron transfer

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Photosynthesis as the major energy conversion process on earth has to use light energy most efficiently. This can only be accomplished via extremely fast initial reaction dynamics which have to compete with ultrafast internal conversion and recombination. How nature has solved this problem has been resolved by femtosecond time resolved spectroscopy on photosynthetic reaction centres. Here the absorption of a photon by the special pair is followed by a linear sequence of electron transport processes, where an electron is transferred over neighbouring chromophores. Reaction rates and energetics of intermediates have been determined and found to be optimised to allow photosynthetic energy conversion with highest quantum efficiency. We present results on native and modified

reaction centres from various species and give an insight into the molecular processes leading to efficient energy conversion.

IL185

Ultrafast dynamics in the Green Fluorescent Protein (GFP) and the GFP chromophore

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The GFP has several properties which make it unique, and of key importance in photobiology. It is an intrinsically fluorescent protein, the visible absorbing chromophore being formed in a cyclisation and oxidation reaction among three amino acid residues. By combining the gene for GFP with that of a target protein it is possible to irreversibly and non-perturbatively label the protein of interest. This has led to the very widespread use of GFP as a fluorescence marker. In addition the excited state of GFP undergoes a proton transfer reaction, the rate of which is a strong function of mutation. This suggests the potential for GFP to act as an *in vivo* sensor, for example for pH or ion concentration.

In this presentation we will consider two aspects of GFP photophysics. First we describe a detailed study of the mechanism of the proton transfer reaction, through ultrafast transient infra-red (TIR) spectroscopy. Using TIR we characterize both the rate of proton transfer and identify the proton acceptor.ⁱ Secondly we investigate the mechanism of the fluorescence enhancement in GFP. A long standing puzzle is that the isolated chromophore of GFP, and denatured GFP are essentially non-fluorescent. We use ultrafast polarization spectroscopy to determine the mechanism of ground state recovery, and fluorescence up-conversion to characterize excited state dynamics. Both are studied as a function of medium viscosity and polarity. These data permit some speculation on the mechanism of the fluorescence enhancement in GFP.^{ii,iii}

ⁱ D. Stoner-Ma, A. A. Jaye, P. Matousek, M. Towrie, S. R. Meech and P. J. Tonge 'Observation of Excited State Proton Transfer in Green Fluorescent Protein Using Ultrafast Vibrational Spectroscopy' *J. Amer. Chem. Soc.*, 2005, *127*, 2864 – 2865

ⁱⁱ K. L. Litvinenko, G. F. White, S. R. Meech, D. L. Andrews and A. J. Thomson 'Multiphoton Excited Luminescence Of Tb^{3+} Bound To Transferrin: A Novel Means Of Intra-Cellular Imaging' *Photochem Photobiol Sci.* 2004, *3*, 47 – 55

ⁱⁱⁱ D. Mandal, T. Tahara and S. R. Meech 'Excited State Dynamics of The Green Fluorescent Protein Chromophore' *J. Phys. Chem. B* 2004, *108*, 1102 – 1108

IL186

Ultrafast photo-isomerization of protonated retinal Schiff bases in different environments

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The photo-isomerization of the retinal cation (PSBR) has been studied in bacteriorhodopsin using a novel VIS-pump/UV-probe scheme. The 80 fs UV-probe pulses measure the bleach signal of residues W86 and W182 as a response to the photo-induced retinal dipole moment change. The latter has thus been followed in real time and evidence for a progressive and unexpectedly large dipole moment increase occurring on a 150 fs time scale is obtained. This finding is discussed in light of the catalytic action of the protein environment promoting isomerization on a 500 fs time scale. We also report on the excited state dynamics of the PSBR in different protic and aprotic solvents at room temperature probed by broadband fluorescence up-conversion with 120 fs time resolution¹. The spectral and temporal signatures of reactive and non-reactive pathways are identified. It is shown that in these loose

environments, solvent polarity and viscosity do not influence the isomerization speed significantly.

ⁱ G. Zgrablić, K. Voitchovsky, M. Kindermann, S. Haacke, M. Chergui, *Biophys. J.* 88, 2779 (2005).

IL187

Fast molecular processes in *Blepharisma japonicum*'s light perception

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The primary phototransduction steps of *Blepharisma japonicum*, a ciliated protozoan exhibiting a strong step-up photophobic response upon illumination, is probed by subpicosecond transient absorption spectroscopy. The photoreceptor of the light-adapted form of the cell is a chromophore-protein complex, composed of a hypericin-like chromophore, oxyblepharismine, non covalently bound to a 200 kDa protein. The chromoprotein was extracted by phosphate-concentration-step chromatography on a hydroxyapatite column but the apoprotein structure has not yet been determined. The primary excited-state reactivity of the chromophore-protein complex was found to differ markedly from that of the isolated chromophore in solution. A strong and specific biexponential decay of a new species formed in the sub-picosecond regime was observed. This deactivation channel was tentatively attributed to an ultrafast intermolecular electron transfer from the excited chromophore to the surrounding protein. We also observed that part of excited chromophore-protein complex population does not follow this reactive pathway and behaves like the free chromophore, possibly because of an unfavourable orientation of the chromophore within the protein pocket.

OC188

Electronic structure, spectroscopy, ultrafast dynamics and aerobic reactivity of trichochromes: natural components of pheomelanins

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The trichochromes are a class of small molecules present in pheomelanin (the red melanin) and absent in eumelanin (the black melanin). Herein decarboxy-trichochrome C (dTTC) and trichochrome F (TF) are examined. Both trichochromes are characterized by a visible absorption band, which is shown to be the result of overlapping transitions of the *cis* and *trans* isomer. The temperature dependence of the absorption spectrum of TF provides the relative extinction coefficients of the two isomers, the temperature-dependence of the equilibrium between the two isomers, from which the enthalpy and entropy differences are obtained. The temperature dependence of the absorption spectrum of dTTC is more complicated and is attributed to the presence of equilibria between the enol and keto forms of the molecule. These results are consistent with the calculated energies of the isomers (both keto and enol forms) using density function theory and a continuum solvation model. Pump-probe optical experiments reveal efficient and nearly complete ground state recovery within a few picosecond of excitation. Aerobic reactivity measurements revealed no detectable production of ¹O₂, and low yield of O₂. DNA nicking assays demonstrated a low level of light-induced aerobic activity of dTTC. Both trichochromes are, however, efficient quenchers of ¹O₂, exhibiting a bimolecular rate constant comparable to vitamin C. These results suggest that trichochromes serve a protective role in pheomelanin pigments.

OC189

Structural characterisation of the fucoxanthin chlorophyll-a/c protein complexes using resonance Raman spectroscopy: the effect on light harvesting and energy transfer dynamics

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Fucoxanthin chlorophyll-a/c protein (FCP), from *Cyclotella meneghiniana*, is studied using Resonance Raman (RR) spectroscopy to fill in the paucity of structural information about these heterokontae algae that possess a similar photosynthetic cycle to the other, more extensively studied, eukaryotic thylakoid systems. The optimisation of the light-absorbing capabilities of these marine organisms, that extends into the blue-green range, depends intimately on the structure and organisation of the bound pigments. Therefore, the vibrational signature of these systems would provide structural information crucial to understand the cascade of ultrafast photophysical events that occur in these proteins.

The FCP complexes studied here include two components extracted by gel filtration with that contain differing amounts of trimers and oligomers. The RR spectra of both complexes reveal two main forms of fucoxanthin, when exciting between 560 and 476 nm, that can be correlated to the red form involved in energy transfer to chlorophyll-a and the blue-absorbing form involved in energy transfer to other fucoxanthins lower in the energy ladder. The effect on the C=C stretching mode, particularly on excitation above 514 nm, is evident in these red fucoxanthins, and interestingly, also shows evidence for their previously discerned charge-transfer character. The RR spectra of the chlorophylls are rich with structural information about the tetrapyrrole macrocycles, e.g., their co-ordination state, and allows the differentiation of distinct populations both within a given complex and between them, the effects being most pronounced for chlorophyll-c. This work describes the fucoxanthins and chlorophylls at a molecular level and delineates their roles as lightharvestors and conduits for energy transfer. Along with the structural information about the species responsible for the initial ultrafast response, the structural changes that occur within 10's of ns shall also be presented.

OC190

Observation of sub-100 ps conformational changes in photolyzed carbonmonoxy-myoglobin probed by time-resolved circular dichroism

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Conformational changes in proteins, which are known to play a paramount role in biophysical processes, are attracting much attention. For example, the change in carboxy-myoglobin (MbCO) after dissociation of the CO has recently been observed with a 100 ps time-resolution in a time-resolved X-Ray experiment. Shorter time resolution is however out of reach of such experiments. In order to investigate these processes on an ultrashort timescale, we have set up a time-resolved circular dichroism (CD) experiment in MbCO. The principle of the experiment is the following: after excitation with a pump beam, the CO-heme link breaks and a deoxy-heme structure appears very rapidly (< 1 ps). As the heme CD in the Soret region is very sensitive to the geometrical arrangement of the surrounding aromatic residues, measuring the change in the CD spectrum with time allows one to gain insight into the first steps of these conformational changes.

The experiment is carried out on a 230 μM, pH 8.0 MbCO sample excited with a 400 nm pulse. The CD is measured across the Soret band as a function of time with a sub-picosecond resolution. After the initial drop in the CD due to the instantaneous electronic change of the heme, we observe a variation of the signal on a sub-100 picosecond timescale. In order to analyze these results, we have developed a calculation after Applequist's normal mode CD theory. Calculation of the contribution of the main residues to the rotational

strength allows us to assign the observed signal to the stress provoked on the proximal histidine by the heme doming. This stress vanishes on a 100 ps timescale as the F-helix relaxes to its steady-state position. Extension of this experiment toward the far ultraviolet will provide a promising technique to investigate elementary changes in the secondary structure of proteins.

PL191

Photoprotection and photorestitution of cultural heritage

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Optics and more recently lasers have entered with many specific contributions the set of technologies employed by conservators and restorers in the everyday fight against the many deterioration dangers. Photons in fact may provide ideal not invasive investigation tools in order to analyse the state of conservation of the material, or the atomic and molecular composition, to detect the presence of structural defects, to individuate traces left by the author, to certificate proofs of authenticity and many other tasks. On the other hand a high intensity photons flux (as is the case of laser emission) may also induce irreversible changes or removal of the material, becoming new restoration techniques. For example welding and cleaning, two very delicate phases for the recovery of deteriorated artworks, may be carried out by a laser with a precision and control not obtainable by other methods. After more than thirty years of development and validation studies nowadays lasers and optoelectronic techniques are widely accepted by conservation institutions in Europe, where the importance to preserve our cultural heritage has brought to successful projects and a number of diagnostics and restoration instruments have demonstrated their value in solving crucial problems. Several renowned masterpieces have gained an acceptable state of conservation due to these techniques, which are continuously spreading in Europe. The scientific and technology cooperation COST Action G7 "Artworks conservation by laser" is carrying out a dissemination plan since the year 2000, promoting best practices and stimulating new applications. The results of this networking activity will be described in details, showing how in this field laser technology became a professional tool in the hand of restorers.

IL192

Regulation of UVB-induced photomorphogenesis in *Arabidopsis*

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The light environment is a key factor that governs a multitude of developmental processes during the entire life cycle of plants. Part of the incident sunlight encompasses a segment of the UVB region (280-320 nm) that is not entirely absorbed by the ozone layer in the stratosphere of the Earth. This fraction of the solar radiation that inevitably reaches the sessile plants is not merely an environmental stress but can also cause morphogenic effects through molecularly yet unidentified UVB photoreceptor(s). The key photomorphogenesis regulator ELONGATED HYPOCOTYL 5 (HY5), a bZIP transcription factor responsible for light-responsive gene expression, is required to signal the perception of UVB. This indicates the use of shared components in morphogenic responses to UVB and visible light. Data will be presented describing regulatory functions that contrast between UVB signalling and the well-investigated dark-to-light transition, demonstrating a novel mode of action employed in the plants' photomorphogenic response to UVB radiation.

IL193

UV-B signalling and protection in *Arabidopsis*

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Exposure of plants to high doses of UV-B radiation (280-320 nm) causes tissue necrosis and induces stress responses in part through activation of pathogen defence and wound signalling pathways. In contrast, exposure to low, physiological levels of UV-B has numerous regulatory effects on plant morphology, development, physiology and biochemical composition. Importantly, low doses of UV-B promote the expression of genes involved in UV-B protection, including those concerned with the production of UV-absorbing phenolic compounds, antioxidant defences and DNA repair. However, despite the importance of these protective responses, remarkably little is known about the underlying mechanisms of UV-B perception and signal transduction. To address this question we have used several approaches, mainly focused on the regulation of transcription of the gene encoding chalcone synthase (CHS) in *Arabidopsis*. CHS is a key enzyme in the biosynthesis of the UV-protective flavonoids and CHS transcription is induced by low fluence rates of UV-B in a pathway distinct to the stress response pathways. We have characterised the UV-B signalling pathway regulating CHS expression in leaf tissue and identified regulators involved in the UV-B induction of CHS transcription. In addition, we have identified mutants altered in CHS induction by UV-B. This presentation will show how our research is advancing understanding of UV-B signal transduction and UV-B protection.

IL194

Impact of UV-B on maize

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Given their sessile growth habit and requirement for sunlight in photosynthesis, land plants are inevitably exposed to UV-B. Plants cope with this environmental stress through various shielding strategies (such as synthesis of sunscreen pigments) and by repair of damage. Using transcriptome profiling of lines varying in sunscreen pigment content we have confirmed several known UV-B stimulated processes and identified new pathways and processes that respond to UV-B radiation in terms of gene expression in a dosage-dependent manner. We have defined gene expression changes in directly irradiated and shielded organs. Translation-associated factors are a major class of responsive genes in irradiated tissues, and we demonstrate that UV-B crosslinks specific ribosomal proteins to RNA and propose that new ribosomes must be synthesized to restore protein synthesis capacity. Using maize landraces from high altitudes we have identified acclimations and adaptations to increased UV-B fluence in the natural environment including flavone sunscreen synthesis in leaves and expression of a suite of likely chromatin-associated factors. RNAi knockdown lines for four such chromatin-associated factors exhibit hypersensitivity to UV-B including sunburning and ultimately necrosis. Our long-term goal is to determine how UV-B radiation reactivates silenced Mutator transposons of maize; reactivation occurs in both directly irradiated tissues (where DNA damage occurs) and in fully shielded tissues. Finding chromatin remodeling factors involved in UV-B responses is a clue to the underlying mechanism of transposon activation. Supported by a grant from the U. S. Department of Agriculture.

IL195**MAP kinases at the crossroads of UV-B and biotic stress signaling pathways**

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Plants in the field are often exposed to several environmental stresses at the same time and the outcome is difficult to predict. We have investigated the responses of tomato plants to wound signals and ultraviolet-B (UV-B) radiation. When plants are wounded by herbivorous insects, they respond with the de-novo synthesis of defense proteins such as proteinase inhibitors (PIs). These proteins inhibit digestive proteases in the intestines of the insects. UV-B irradiation of tomato seedlings does not result in PI synthesis. However, when plants were wounded and shortly thereafter irradiated with UV-B, PI synthesis was strongly potentiated. We are interested in the molecular mechanisms underlying this synergistic interaction. We identified three levels of signal transduction common to UV-B and wounding. 1) The wound signaling peptide systemin and UV-B both induce alkalization of the growth medium in suspension-cultured cells. The alkalization response is mediated by the plasma membrane proton ATPase and essential for systemin-induced PI synthesis. 2) Mitogen-activated protein kinases (MAPKs) are universal eukaryotic signalling relays. We have shown that the tomato MAPKs LeMPK1 and LeMPK2 respond to wounding, systemin, oligosaccharide elicitors, and UV-B radiation. A third MAPK, LeMPK3, is activated only by wounding and UV-B. Gene silencing experiments demonstrated that LeMPK1 and 2 are essential for the systemin-induced defense response. These MAPKs are central convergence points for many stress signals raising the question as to how signal specificity can be achieved via highly promiscuous signal transducers. Our work indicates several possibilities including MAPK activation kinetics and combination of different sets of active kinases. These findings are consistent with the synergistic effects of UV-B and wounding, but also with additional signal-specific responses. 3) High UV-B fluxes are perceived in an unspecific manner and result in generation of reactive oxygen species which can assume a signaling function. In contrast, systemin activates LeMPK1/2 via the systemin receptor SR160, a membrane-spanning LRR receptor kinase. Our experiments indicate that UV-B co-opts SR160 in a ligand-independent manner similar to the activation of growth factor receptors by UV-B in animal cells. Unspecific activation of cell surface receptors by UV-B would have far-reaching implications for UV-B and oxidative stress signaling and is consistent with known overlaps between the systemin/wound response and the UV-B response.

This work is supported by grants from the National Science Foundation, USA (Award #IOB-0321453 and MCB-0418890).

OC196**The role of the FtsH and DegP proteases in the repair of Photosystem II after damage by UV-B radiation in *Synechocystis* 6803**I. Vass¹, O. Cheregi¹, C. Sicora¹, P.B. Kos¹, P.J. Nixon²¹Institute of Plant Biology, Biological Research Center, Szeged, Hungary; ²Department of Biological Sciences, Imperial College London, Biochemistry Building, South Kensington campus, London, SW7 2AZ, UK

We have investigated the role of the FtsH and DegP protease homologues in the repair of UV-damaged PSII in *Synechocystis* 6803 cells. Inactivation of the three DegP genes, which encode DegP protease homologues, had no significant effect on the UV-B induced loss of oxygen evolving activity and its subsequent recovery. In contrast, inactivation of the slr0228 gene, which encodes an FtsH protease homologue, accelerated the UV-B induced loss of oxygen evolving activity, as well as its recovery in visible light following the UV-B treatment as compared to the WT cells. In the presence of the protein synthesis inhibitor lincomycin the rate of UV-B induced inhibition of oxygen evolution was

significantly accelerated in the WT, but not in the Δ FtsH mutant. These data show that PSII repair is blocked in the absence of the FtsH protease both under UV illumination and in visible light following the UV-B exposure. The loss of the D1 protein during UV-B illumination is slowed down in the Δ FtsH mutant as compared to the WT cells both in the presence and absence of lincomycin, showing that D1 degradation is retarded in the Δ FtsH mutant. In contrast, the loss of the D1 protein was not affected by deletion of DegP. From these data we conclude that the FtsH protease is involved in the degradation of the UV-damaged D1 protein, which is required for PSII repair via *de novo* synthesis and incorporation of new D1 copies into the PSII reaction centers. In contrast, the DegP protease does not seem to be involved in D1 turnover and PSII repair under UV-B light.

OC197**Formulation of a thermosetting hydrogel of hexylaminolevulinat targeting Barrett's oesophagus**S. Collaud¹, F. Evangelisti¹, L. James¹, Q. Peng², R. Gurny¹, N. Lange¹¹Laboratory of Pharmaceutical Technology and Biopharmacy, School of Pharmaceutical Sciences Geneva-Lausanne, University of Geneva, Switzerland; ²Department of Pathology, The Norwegian Radium Hospital, University of Oslo, Norway

Barrett's oesophagus represents a pre-cancerous condition that has a specifically increased risk for the development of adenocarcinoma of the oesophagus. 5-ALA-mediated fluorescence photodetection has shown to be helpful to visualize dysplastic lesions or non-evident early cancer in Barrett's oesophagus. Although the sensitivity of this new diagnostic tool is excellent, its selectivity has shown to be inferior by 20% to the standard procedure of random biopsies. This might be due to the systemic administration of 5-ALA leading to the characteristic red fluorescence of PpIX in normal structures, thereby diminishing the total contrast. Topical application of hexylaminolevulinat (HAL) to Barrett's oesophagus could represent an advantageous alternative in terms of adverse side effects, application time, as well as contrast and PpIX fluorescence intensity in the target tissue. Thus, hydrophilic thermosetting formulations based on poloxamer 407 have been developed. Such formulations are characterized by a pour point at which the liquid hydrogel (sol) turned suddenly to solid (gel). This transition occurs at a specific temperature which is mainly influenced by the concentration of gel former in the formulation. Therefore, an optimised formulation ingested as a liquid would gelify on the target area, thereby increasing contact time between the hydrogel and the mucosa, thus improving mucosal delivery of HAL. A simple *in vitro* model has been developed in our lab to simulate the oesophagus and test various candidates having different pour points. The present study shows that when administered at 20°C, the formulations with a sol-gel transition temperature of about 25°C are optimal to target Barrett's oesophagus since a maximal amount of hydrogel reaches the area of interest. The optimised formulation has been also evaluated with respect to stability, *in vitro* release of HAL and porphyrin formation in nude mice.

OC198**On the importance of light delivery and intensity measurements during ALA PDT in otorhinolaryngology**T. Vidóczy¹, P. Baranyai¹, L. Csokonai Vitéz², B. Horváth²¹Photoscience Laboratory, Department of Physical Chemistry of the Budapest University of Technology and Economics, and of the Chemical Research Center, Budapest, Hungary; ²National Medical Center, Department of Otorhinolaryngology, Budapest, Hungary

Light delivery and measurement of the actual light intensity on the illuminated spot is crucial for the effectiveness of 5-aminolevulinic acid induced photodynamic therapy (ALA PDT). Hard-to-reach areas in otorhinolaryngology, like the larynx, the pharynx or the

sinus, are especially problematic both from the point of view of light delivery and measuring the delivered light intensity. The primary photosensitizer in ALA PDT is protoporphyrin IX, its last absorption band in the red spectral region is centered at 635 nm. During PDT, however, protoporphyrin is partly converted to a photoproduct, which is a sensitizer in its own right, and the last absorption band in the red spectral region of this photoproduct is centered at 675 nm. Therefore applying a broadband light source (using radiation between 600 – 700 nm) is advantageous in ALA PDT, since it can activate both protoporphyrin and its photoproduct. We give an example of the general setup of a broadband light source based on a high pressure xenon lamp, providing illumination in selectable wavelength ranges through a fiber bundle. We show the design of special rigid handpieces to be applied in the mouth, and others, useful down to the vocal cords, together with illustrative examples of the light intensity distribution emerging at the output of these devices.

Applying PDT in the oral cavity, or in the sinus calls for illumination of irregularly shaped areas. Therefore estimation of the local light intensity based on the light intensity emerging from the fiber tip would be rather inaccurate, so it has to be determined directly. We show arrangements capable to measure local light intensities in the typical power range applied in PDT (10 – 300 mW.cm⁻²) even in hard-to-reach areas, where scattered light may contribute significantly to the total intensity. These devices can be used during PDT, enabling the accurate calculation of treatment time, even in the case of light intensity variation during treatment.

OC199

ALA-PDT effect on adhesion and cytoskeleton of the cultured human carcinoma and glioma cells

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Sublethal ALA PDT inhibited both: attachment of carcinoma WiDr cells to the plastic substratum and trypsin-induced cell detachment of carcinoma WiDr and glioma D54Mg cells. ALA PDT of WiDr cells that form densely packed colonies increased intracellular space; induced formation of actin cortex and lamellipodia between cells inside colonies, increased numbers of focal contacts containing $\alpha_v\beta_3$ integrin and actin stress fibers in some but not all cells. It did not change microtubule cytoskeleton and E-cadherin distribution. The same photosensitization of D54Mg cells, which grow separately and communicate by protrusions, caused loss of fibrillar actin structures in growth cones, retraction of protrusions, and surface blebbing in some cells. No changes in $\alpha_v\beta_3$ integrin and E-cadherin distribution were observed. The experiments with cytoskeleton inhibitors cytochalasin D, colchicine or taxol showed involvement of microfilaments and microtubules in ALA PDT effect on trypsin-induced cell detachment. Some signal transduction processes are suggested to be involved in ALA-PDT-induced changes in cytoskeleton, cell shape and adhesion.

OC200

ALA-induced endogenous porphyrins in arthritic rabbit knee tissues: a spectroscopic study

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The inflamed synovium of rheumatoid arthritis exhibits many features typical for neoplastic tissue implying that the photodynamic therapy might be an efficient modality for chronic polyarthritis. A rabbit model of antigen-induced rheumatoid arthritis was used to study the accumulation of endogenously produced porphyrins after administration of 5-aminolevulinic acid (ALA) by

means of a fiber optics based fluorescence spectrometer under excitation at around 405 nm.

The slight increase of porphyrin fluorescence had been already detected in the autofluorescence spectra of inflamed tissues before the external sensitisation. In spite of the way, intravenously or intra-articularly, ALA was injected to the experimental animals, the highest fluorescence intensity of endogenously produced porphyrins measuring from the surface of the skin above the synovium was detected in the tissues of the inflamed joints 1-3 hours after the administration of ALA. Besides, the application of ALA had a systemic sensitising effect on the whole organism of rabbits. A rapid removal of porphyrins from the sensitized tissues was observed and a weak fluorescence signal of porphyrins remained only in the spectra of arthritic knees 24 hours after ALA injection.

Comparative fluorescence measurements performed *ex vivo* on the tissue specimens taken from the inflamed and control joints at various times revealed that not only the amount of porphyrins accumulated in the synovium, cartilage, tendon, skin, and muscle tissues was different, but also their relative composition, which comprised PpIX as well as more hydrophilic porphyrins, such as uro- and coproporphyrins. The factors affecting the specific distribution of endogenously produced porphyrins in different tissues and its implication for the photodynamic therapy are discussed.

OC201

Meta-tetra(hydroxyphenyl)chlorin (mTHPC) aggregation state and interaction with plasma albumin

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Present study addresses the aggregation state of mTHPC and its interaction with serum albumin. The samples of mTHPC (3×10^{-6} M) solutions were incubated in the presence of bovine serum albumin (BSA, 10 mg/mL) for 1 hour at 37°C and eluted through the column filled with gel Sephadex G-100. mTHPC elution profiles demonstrated two main bands: the first weakly fluorescent and a second one with a fluorescence quantum yield several times higher. Upon addition of surfactant to each fraction we observed a very large increase in sensitizer fluorescence intensity in the first with little change in the fluorescence of the second. Gel-filtration experiments with mTHPC in solutions of monomeric BSA revealed that non-fluorescent mTHPC in the first elution peak is not bound to BSA molecules. Fluorescence, absorbance and resonance light scattering (RLS) studies have showed that the first chromatographic fraction contains large aggregates of mTHPC composed of hundreds of sensitizer molecules. With the aim to investigate the size distributions of mTHPC aggregates in various media we further used photon correlation spectroscopy (PCS) technique. The hydrodynamic diameters (D_H) and the values of polydispersity index (PI, distribution width) varied widely in phosphate buffered saline (PBS) and PBS containing various concentrations of BSA. Just after injection of mTHPC (3×10^{-6} M) in PBS solutions the average D_H values of its aggregates was 440 nm (PI = 25 %). The values of D_H increase with increasing photosensitizer concentration and decrease with increasing ethanol content. The use of PBS-BSA solution leads to formation of considerably smaller aggregates with D_H values 4-5 times less compared to PBS only. With increasing incubation time in PBS-BSA solution the D_H values of aggregates decrease. This complex inter-relationship between photosensitizer aggregation state and interaction with plasma albumin should be considered when assessing sensitizer biodistribution.

OC202**The effect of Photofrin-PhotoDynamic Therapy (PDT) on mechanisms of cell death in EMT6 murine cells**

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The induction of tumour-specific immunity is essential for achieving long-term therapeutic benefit after PDT, and this might be influenced by the mode of cell death induced (apoptosis/necrosis), as apoptotic cells can have anti-inflammatory effects. This study investigated the induction of cell death by Photofrin-PDT *in vitro* and *in vivo* using an EMT6 mammary adenocarcinoma murine model. Cells were incubated for 24 hr with 1µg/ml Photofrin, followed by illumination at 3J/cm² using a 635nm laser. Cytotoxicity was determined by flow cytometry using the Annexin V-FITC/PI assay between 30 min and 24 hr post-PDT. The induction of cell death by PDT was also examined in Balb/c mice bearing 350mm³ subcutaneous EMT6 tumours. Photofrin (10mg/kg iv) was administered and tumours were illuminated (100J/cm²) with the laser 24 hr thereafter. Tumours were resected 2 hr post-PDT and sections cut from formalin-fixed, paraffin-embedded tissue blocks were stained with haematoxylin and eosin for histological examination. A relative increase in necrosis from 67.14% ± 2.39% in the light-treated control to 81.62% ± 5.46 was evident at 30 min following *in vitro* PDT. Annexin V-FITC/PI studies conducted at 24 hr post-PDT confirmed that cell viability further declined from 43.95% ± 3.09 in the light-treated control to 18.20% ± 11.42 with a concomitant increase from 7.99% ± 1.01 to 50.33% ± 19.28 in apoptotic cells. The PDT-treated subcutaneous EMT6 tumours showed signs of necrotic degeneration. The microvasculature displayed no visible damage and the observed neutrophil polymorph accumulation was considered to be a histological feature of acute inflammation. Photofrin-PDT appears to induce an early necrotic response *in vitro* and *in vivo*, which may promote the development of protective immunity. Furthermore, the subsequent induction of apoptosis observed *in vitro* might initiate anti-inflammatory effects and moderate the generation of tumour-specific immunity. Research is funded by AICR.

OC203**Effect of hypericin on intracellular localization of PKC in U-87 MG human glioma cells: competitive binding of Hyp and PMA to C1B sub-domain of PKC**

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Flow-cytometry experiments have shown that hypericin (Hyp) phototoxicity induces both, apoptosis and necrosis of U-87 MG human glioma cells in a concentration- and light dose-dependent way. Fluorescence imaging technique was used to monitor intracellular localization of Protein kinase C (PKC) in U-87 MG human glioma cells. It is shown that PKC localization, which reflects its activity is influenced by Hyp and this influence is different from that observed for phorbol 12-myristate 13-acetate (PMA) which acts as PKC activator. Fluorescence binding experiments were realized to determine the binding constant of Hyp to PKC which is compared with that already determined for PMA. Finally, molecular modeling was used to compare structural models of the PKC- α , - β , - γ /Hyp (C1B domain/Hyp) and the PKC/PMA (C1B domain/PMA) complexes. The influence of Hyp and/or PMA on PKC translocation in U-87 MG cells, co-localization fluorescence pattern of Hyp and PKC, the higher binding affinity

of Hyp to PKC in comparison with PMA, as well as the binding mode of Hyp to C1B domain of PKC suggested by molecular modeling support the hypothesis, that Hyp competitively binds the same binding site in PKC as PMA.

Acknowledgment: this work was supported by the Slovak Science and Technology Assistance Agency, grant No. APVT-20-036104 and by the Scientific Grant Agency of the Ministry of Education of Slovak Republic grant No. 1/2278/05.

OC204**Enhanced selectivity of chemo and photochemotherapeutic agents via protease mediated drug delivery**

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Chemotherapeutic agents show relative reactivity towards neoplastic tissue, but little or no selectivity towards tumour tissues. The opposite is true for photochemotherapeutic agents, which are generally retained longer in diseased tissues. In order to combine both advantages in one methodology, one can exploit the fact that invading tumours often show an overexpression of proteolytic enzymes at the invading tumour front. Thus a new series of potential anti tumour drugs have been made that consists of three components: the active component (either a chemo or a photochemotherapeutic agent), a solubilizer, and an amino acid sequence linker that is susceptible to cleavage by the overexpressed proteolytic enzymes. Upon such cleavage, the anti-tumour agent experiences a rapid change in solubility, thus being effectively "trapped" at the tumour's invading front. This novel, patented process (US patent No. 5,618,790) has the potential for selective, clean destruction of the tumour front, with greatly reduced risk of recurrence and minor side effects. Our first tri-component drug, used to demonstrate proof of concept, consists of mesoporphyrin IX as the active photochemotherapeutic agent, linked to tetralysine solubilizers via the following amino acid sequences: Gly-Pro-Leu-Gly-Pro-Ala. The linking components contain the Leu-Gly linkage, which is susceptible to breakage by proteolytic enzymes. Tri-component prodrugs with doxorubicin as the active agent also show enhanced anti-tumour activity [A.M. Manswour et al, (2003) Cancer Res. 63, 4062-4066; V. Dubois et al, (2002) Cancer Res. 62, 2327-2331].

OC205**FLIM and SLIM for molecular imaging in PDT**

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Various problems arising during molecular imaging of different fluorophores and metabolites used in photodynamic therapy could be circumvented by focusing on time-resolved detection. For this, an interesting new method is time-correlated single photon counting, where a time-to-amplitude converter determines the temporal position and a scanning interface connected to the scanning unit of a laser microscope determines the spatial location of a signal. In combination with spectral resolved detection (spectral lifetime imaging, SLIM) the set-up achieves the features of highly sophisticated lifetime imaging systems.

The photoactive substance on which 5-ALA PDT is based, is protoporphyrine IX which is synthesized in mitochondria. Alternatively, other metabolites from 5-ALA could be involved. Subcellular differentiation of those metabolites without extensive extraction procedures is not trivial, because of highly overlapping spectral properties. Measuring the fluorescence lifetime on a subcellular level could be a successful alternative.

To record lifetime images a setup consisting on a laser scanning microscope equipped with detection units for SLIM (SPC-830 with 16 routing channels (Becker & Hickl) and ps diode lasers for short-

pulsed excitation was implemented. The time-resolved fluorescence characteristics of 5-ALA metabolites were investigated. The lifetimes were best fitted by a bi exponential fitting routine. Different lifetimes could be found in different cell compartments. During illumination, the lifetimes decreased significantly. Different metabolites of 5-ALA could be correlated with different fluorescence lifetimes. In addition cells were coincubated with the nuclear staining dye DAPI, in order to investigate the cell cycle. In contrast to ALA, the lifetime of DAPI, which was best fitted mono exponentially did not change during photobleaching.

This work is supported by the ministry of economics Baden-Württemberg from a grant of the Landesstiftung

OC206

Analyse of monolayer cells by MALDI-TOF/MS - Applications for photodynamic therapy

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Among different treatments of cancer, photodynamic therapy (PDT) is an original, recent and efficient modality to reduce and even to induce tumors destruction. These studies consist to elucidate PDT mechanisms by studying colo HT29 cells with Mass Spectrometry technique. A new sampling system which is a glass slide, was used in order to follow the *in situ* photobleaching of foscan[®]. The present work shows a method to control the *in situ* behavior of photosensitizer by MALDI-MS. It gives the possibility to realize the analyses with different laser wavelengths. Methylen blue was used to localize these cells and to control the good adhesion of cells on support. Cells were then impregnated by *m*-THPC at three concentrations (20, 10 and 5 µg/mL). In order to verify that detected *m*-THPC corresponded to cellular *m*-THPC, the solutions of washing were analyzed by MALDI-TOF/MS. These experiments show that more the signal fluence is high, weaker the *m*-THPC signal is. We noticed also that proteins have an influence on the photobleaching of *m*-THPC.

Further to these studies, we have analyzed two other cellular types (MCF 7 and fibroblasts) with this original sampling protocol. Their proteinic finger printings were characterized. These studies should be completed by conventionnal proteomic analyses (electrophoresis). A protocol will be discussed based on instrumental development (coherent laser in TOF MS source) and on the advantages that FTICR/MS technique can bring in proteomic field (high resolution and accurate mass).

OC207

Interaction of UVC/UVB radiation with DNA simple and double helices: from photon absorption to photodamage

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Direct absorption of UV radiation by DNA bases is known to trigger photochemical reactions which may lead to carcinogenic mutations. Although the major lesions are well characterized, the physicochemical processes which precede their formation remain unknown. By studying model DNA simple and double helices, we have shed some light on three different aspects related to the excited states populated by photon absorption, energy transfer among bases and the dynamics of thymine dimer formation.

The excited states of (dA)n.(dT)n and (dAdT)n.(dAdT)n reached by UV absorption were studied theoretically, combining exciton theory, molecular dynamics and quantum chemistry data. Most of the excited states were found to extend over a few bases. The

simulated absorption spectra are only slightly shifted with respect to those of noninteracting bases, in agreement with experimental observations. The states with larger spatial extent are located close to the maximum of the absorption spectrum.

The above double helices were studied by fluorescence time-resolved spectroscopy, from the femtosecond to the nanosecond timescales. Energy transfer was evidenced by the anisotropy decay occurring at times shorter than one picosecond. The sequence independence of the anisotropy decay indicates that energy transfer involves delocalized excited states.

At longer times, the formation of thymine dimers was studied by flash photolysis. In (dT)₂₀, the (6-4) adduct is formed within 4 ns via a reactive intermediate. The formation of cyclobutane dimers is faster than 200 ns. This is the first observation of the time-scales associated with the DNA photoreactions.

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OC208

Photocaged radicals for the studies of oxidative DNA damage

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Reactive oxygen species (ROS) are produced in both endogenous and exogenous processes including ionizing radiation, and ROS-induced DNA damage has been implicated in the pathogenesis of a number of human diseases including cancer and ageing. Here in the presentation I will discuss the synthesis of photolabile precursors for radicals formed on pyrimidine nucleosides and their applications for the studies of oxidative DNA damage. In particular we are interested in the type of damage where neighboring nucleobases in the same DNA strand are covalently bonded. By using these photolytically generated radicals, we were able to isolate several novel intrastrand crosslink lesions and establish their structures by mass spectrometric and NMR spectroscopic techniques. In addition, we employed LC-MS/MS and demonstrated the formation of those lesions in duplex DNA by γ irradiation under both aerobic and anaerobic conditions. The photochemically generated radical also enabled us to obtain oligodeoxynucleotide substrates containing a structurely defined intrastrand crosslink lesion. With these substrates, we further examined the mutagenic properties of those lesions by *in vitro* replication studies. Our results showed that the intrastrand crosslink lesions, if not repaired, can be mutagenic.

OC209

Application of UV laser photochemistry to the studies of histone-DNA interactions and transcription factor binding to nucleosomes

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Protein-DNA complexes, including nucleosomes, play a key role in the regulation of major cellular processes such as chromosome

assembly, gene expression, DNA replication and repair. High-intensity UV laser ($\lambda \sim 260$ nm) mediated-"footprinting" and DNA-protein crosslinking represent powerful approaches to investigate transient protein-DNA interactions under both *in vitro* and *in situ* conditions. The generation of guanine radical cations involves biphotonic ionization processes associated with energy and charge migration phenomena (Douki et al, 2004; Angelov et al, 2004a). This explains why the latter approach can be used as a highly sensitive probe of DNA conformational changes occurring upon protein binding. This allows a single UV laser pulse to be used in high-resolution dynamic studies of interactions between proteins and DNA, through "photofootprinting" studies. In addition, UV laser irradiation of protein-DNA complexes gives rise to the formation of covalent adducts with a high quantum yield exceeding up to 50-100 times that obtained using conventional low-intensity sources.

A few relevant examples of application of these novel laser-based methods to the study of nucleoprotein complexes are provided. These include the complexes between the NF- κ B transcription factors with DNA within positioned nucleosomes. Inspection of crystal structures of the NF- κ B-DNA complexes and nucleosomes suggest incompatibility of formation of NF- κ B - nucleosomes complexes. Surprisingly, we found little inhibition effects of the nucleosomes structure independently on the position of the binding sequence (Angelov et al, 2004b).

The involvement of histone NH₂-tails in the plasticity of nucleosomes was investigated by UV laser crosslinking associated with chromatin immunoprecipitation (laser ChIP) assays. This enabled to demonstrate that histone NH₂-tails interact with linker DNA. Interestingly, these interactions were found to persist upon histone hyperacetylation, transcription factor binding and nucleosomes remodelling by SWI/SNF complexes.

Acknowledgements: this work was supported by the European Commission through network grant *MCRTN-CT-2003-505086 (CLUSTOXDNA)*.

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OC210

Lipid hydroperoxides as an endogenous precursor of singlet molecular oxygen in the presence of metal ions and other reactive species

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Lipid hydroperoxides (LOOH) are the primary products of photosensitized oxidation of membrane lipids. Their decomposition is known to generate more reactive and toxic compounds, such as peroxy radicals (LOO[•]). These radicals play an important role in the propagation of lipid peroxidation and may generate singlet molecular oxygen (¹O₂) through the combination of two LOO[•] molecules as described by Russell. In this study we have investigated LOOH, in particular linoleic acid hydroperoxide (LAOOH), as a source of ¹O₂ in the presence of biologically relevant oxidants such as, metal ions, peroxytrite or hypochlorite. The formation of ¹O₂ was clearly demonstrated in the reaction of LAOOH with all the three tested oxidants by detecting: (i) the dimol light emission in the red spectral region ($\lambda > 570$ nm), (ii) the monomol light emission in the near-infrared region ($\lambda = 1270$ nm), (iii) the infrared light emission spectrum, and (iv) the enhancing effect of deuterium oxide and the quenching effect of azide on light emission. Furthermore, the mechanism was studied using LAOOH labeled with 18-oxygen isotope (LA¹⁸O¹⁸OH) and specific ¹O₂ chemical traps in combination with HPLC coupled to mass

spectrometry detection. The results showed the formation of 18-oxygen labeled ¹O₂ (¹⁸[¹O₂]) in the reaction of LA¹⁸O¹⁸OH with the three oxidants, indicating that oxygen atoms in ¹⁸[¹O₂] are derived from the hydroperoxide. Altogether, the obtained evidences lead to the conclusion that LOOH may serve as a potential source of ¹O₂ in biological system and this process may be involved in the mechanism of DNA damage and tumour cell killing promoted by photodynamic therapy. Supported by FAPESP, CNPq, USP and Guggenheim Foundation.

OC211

Singlet oxygen-mediated formation of protein peroxides in cells and its consequences

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Photo-oxidation reactions in cells can damage proteins, lipids, cholesterol, DNA and low-molecular-mass compounds. Proteins are likely to be major targets due to their abundance, but the chemistry and biochemistry of the resulting species are incompletely understood. In this study we have quantified peroxide formation on proteins, lipids and low-molecular-mass substrates in sensitizer-loaded THP-1 (macrophage-like) and MRC5 (fibroblast) cells exposed to visible light. Peroxide concentrations increased with the illumination time, and varied with the sensitizer in the order: Rose Bengal > AlPcS₄ \approx tetrakisporphine \approx hematoporphyrin IX > ZnPcS₄. Peroxide concentrations were enhanced, in some cases, in the presence of D₂O, and decreased by azide, consistent with the mediation of ¹O₂. Quantitation of the various peroxide populations present in these cells showed that Rose Bengal- and tetrakisporphine-loaded cells give higher levels of protein than lipid peroxides, whereas more lipid than protein peroxides were detected with AlPcS₄ and hematoporphyrin IX. This presumably reflects, at least in part, the sub-cellular localization of the sensitizers within cells. Low-molecular-mass peroxides accounted for $\leq 15\%$ of the total peroxide yield; these species may be important in mediating damage to neighbouring organelles and cells. Similar peroxides have been detected on isolated proteins, peptides and amino acids, on exposure to light in the presence of Rose Bengal and O₂. These peroxides are poorly removed by cellular defense systems (both enzymatic and non-enzymatic), consistent with the long half-life of protein peroxides within cells. Protein and peptide peroxides can however inactivate thiol-dependent cellular enzymes (cathepsins, caspases, GAPDH), with damage to the lysosomal cathepsins, which are partially responsible for the proteolytic removal of damaged proteins potentially contributing to the accumulation of these species within cells, and their detrimental effects.

OC212

UVA radiation is highly mutagenic in cells that are unable to repair 7,8-dihydro-8-oxoguanine in *Saccharomyces cerevisiae*

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Ultraviolet A (320-400 nm) radiation constitutes more than 90% of the environmentally relevant solar UV radiation and it has been proposed to have a role in skin cancer and ageing. Due to the popularity of the high-intensity UVA tanning equipment and prolonged periods of sunbathing, the potential deleterious effect of UVA has emerged as a source of concern for public health. Although generally accepted, the impact of DNA damage on the cytotoxic, mutagenic and carcinogenic effect of UVA radiation remains unclear. In the present study, we investigated the sensitivity of a panel of yeast mutants affected in the processing of DNA damage, to the lethal and mutagenic effect of UVA radiation. The data demonstrate that none of the major DNA repair pathways such as base excision repair, nucleotide excision repair, homologous recombination and post-replication repair efficiently

protect yeast from the lethal action of UVA radiation. On the other hand, the results show that the Ogg1 DNA glycosylase efficiently prevents UVA-induced mutagenesis, suggesting the formation of oxidized guanine residues. In addition, using alkaline agarose gel electrophoresis, we show that Fpg-sensitive sites increase with increasing UVA doses, whereas cyclobutane pyrimidine dimers could not be detected. In agreement with the above observations, sequence analysis of UVA-induced Can^R mutations reveals a bias in favor of GC to TA events, when compared to spontaneous or H₂O₂-, UVC- and γ -rays- induced Can^R mutations in the wild-type strain. Taken together, our data point out a major role of oxidative DNA damage, mostly 7,8-dihydro-8-oxoguanine (8-oxoG), in the genotoxicity of UVA radiation in the yeast *Saccharomyces cerevisiae*. Therefore, the capacity of skin cells to repair 8-oxoG may be a key parameter in the mutagenic and carcinogenic effect of UVA radiation in humans. An effective protection of skin would require the use of UVA blocking agents associated with an antioxidant strategy.

OC213

Sensitivity to polychromatic UV-radiation of strains of *Deinococcus radiodurans* differing in their DNA repair capacity

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The ubiquitous bacterium *Deinococcus radiodurans* has been ranged in the group of polyextremophiles, due to its extraordinary resistance to a variety of environmental stress agents, such as ionizing and UV radiation together with organic peroxides and desiccation. Its survivability has been interpreted as the result of an evolutionary process that selected for organisms that could tolerate massive DNA damage. Phylogenetically, *Deinococcus* species represent a lineage at least as old if not older than the rest of the 10 phylogenetic lineages of eubacteria. If this organism already existed in the Archaeal (3.8 to 2.5 Ga ago), it had to cope with a UV radiation environment ($\lambda > 200$ nm) of UV-doses exhibiting a 1000 times higher biological effectiveness than those at present ($\lambda > 295$ nm), due to the lack of an efficient UV-screening by the stratospheric ozone layer, which was built up about 2 Ga ago.

In the current work *D. radiodurans*' response to the simulated UV radiation environment (> 200 nm) of the early Earth as well as to selected spectral ranges of the environmental UV climate on present Earth is reported. The inactivation of the wild type strain R1 of *D. radiodurans* was compared with that of the UV_{254 nm}-sensitive mutants UVS78 *uvrA1 uvsE*, and IR1A *recA*, as well as the UV_{254 nm}-resistant mutant 262 *uvrA2*. In addition to the inactivation studies the DNA photoproducts induced by the different radiation qualities were determined by HPLC-MS/MS. It was found that (i) the spectrum and frequencies of bipyrimidine photoproducts were identical in the wild-type strain R1 and the repair deficient strain UVS78, and (ii) the difference in UV-sensitivities between the wild-type and the repair-deficient strains that was maximal for the full UV-spectrum (> 200 nm), decreased when moving the cut-off towards longer wavelengths, and disappeared completely for the nearest UV (> 315 nm). The data may provide some rationale for a possible evolutionary advantage of a highly efficient DNA repair in the Archaeal. They further suggest, that apart from the bipyrimidine photoproducts in the DNA, additional damage might be induced in the cells by the long wavelength UV radiation (UV-A) which are not amenable to the DNA repair pathways mentioned above.

OC214

Killed But Metabolically Active (KBMA) microbes: a new vaccine paradigm for eliciting effector T cell responses and protective immunity

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Vaccines based on live-attenuated organisms are desirable for their immunologic potency, but the risk of disease precludes their use in many instances. To address this dilemma, we have developed a new class of vaccines based on killed but metabolically active (KBMA) bacteria, which simultaneously takes advantage of the potency of live and the safety of killed vaccines. We removed genes required for nucleotide excision repair (*uvrAB*), rendering microbial-based vaccines exquisitely sensitive to inactivation by photochemical treatment combining psoralen and long wave ultraviolet light. Replication and colony formation of the nucleotide excision repair mutants was blocked by infrequent, randomly distributed psoralen crosslinks, but the resulting bacterial population was able to express its genes, synthesize and secrete proteins. Using the intracellular pathogen *Listeria monocytogenes* (*Lm*) as a model platform, recombinant psoralen inactivated *Lm* Δ *uvrAB* vaccines induced potent CD4⁺ and CD8⁺ T cell responses and protected mice against virus challenge in an infectious disease model and provided therapeutic benefit in a murine cancer model. Microbial KBMA vaccines used alternatively as a recombinant vaccine platform or a modified form of the pathogen itself may have broad use for the treatment of infectious disease and cancer.

OC215

Experimental and theoretical study of the interaction of single-stranded DNA homopolymers and a monomethine cyanine dye: nature of specific binding

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The interaction of dyes with DNA is widely used in analytical chemistry, microscopy, and many other imaging applications. In recent work we have shown that the fluorescence lifetimes of intercalated dyes, particularly PicoGreen[®], can be used to distinguish single- from double-stranded DNA, and ultimately to monitor DNA damageⁱⁱⁱ. Unfortunately, the structure of PicoGreen is proprietary, making it impossible to study it using computational methods. The structure of PicoGreen is known to resemble the monomethine cyanine dye thiazole orange (TO). Several monomethine cyanine dyes have proven useful as sensors for DNA and its structural changes. The nature of the association process is not always clear; this is particularly true of single-stranded DNA. For example, Nygren et al.ⁱⁱⁱ found that TO binds with high affinity as a monomer with poly(dA) in an intercalation complex, and possesses a high fluorescence quantum yield; it was suggested that the larger size of purines offer a larger surface area for hydrophobic interactions.

We have examined the interaction of a TO derivative with DNA homopolymers using steady-state and time-resolved fluorescence spectroscopy, circular dichroism and UV-visible absorption spectroscopy, and computational methods. The calculations, in combination with the spectroscopic data, provide a unique understanding of the interactions between the dye and the homopolymers. Such information can contribute to the design of new dyes that are more effective as selective intercalators.

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OC216

Photoinduced DNA damage by quinolizinium derivatives – Singlet oxygen generation and unusual formation of hydroxyl radicals

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We have observed recently that DNA is damaged upon irradiation in the presence of quinolizinium derivativesⁱ. In this paper, detailed mechanistic investigations of this photo-induced DNA damage by benzo-annelated quinolizinium salts (4aazoniaanthracene derivatives) will be presentedⁱⁱ. Irradiation of 9-bromo[*b*]benzoquinolizinium in the presence of defined double- and single-stranded DNA oligomers under aerobic conditions leads to both frank strand breaks and alkali-labile sites as determined by polyacrylamide gel electrophoresis (PAGE). The extent of the DNA damage increases significantly in D₂O and occurs selectively at guanosine residues. These observations reveal the formation of singlet oxygen (¹O₂) as reactive intermediate, which oxidizes the DNA bases, above all the guanine bases. Further evidence for ¹O₂ formation was obtained from laser-flash spectroscopic investigations, which show intersystem crossing (S₁ to T₁) of the excited states of the parent benzo[*b*]quinolizinium and of the 9-bromo- and 9-amino-substituted derivatives. The resulting triplet state is efficiently quenched by oxygen (k_q > 10⁹ s⁻¹M⁻¹) to yield ¹O₂. Under anaerobic conditions, no significant alkali-labile lesions are observed, but frank strand breaks are induced; however, to lesser extent than under aerobic conditions. The DNA damage is suppressed in the presence of a radical scavenger, namely *t*-BuOH, and hydroxyl radicals are shown to be the reactive intermediates by trapping experiments with terephthalic acid. Moreover, the intercalated acridizinium molecules are not involved in the DNA damage reactions. The intercalated acridizinium salt leads to a primary PET reaction with the DNA bases; however, a fast BET transfer is proposed that regains the dye and the DNA, so that the excited intercalated dye does not contribute significantly to the overall DNA damage.

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OC217

Photoprocesses in densely packed stable stacking aggregates of nucleic bases of candidates for the role of first genetic matrixes

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Stable-stacking aggregates of adenine, adenosine and cytidine have been revealed in water solutions at concentrations of 10⁻² – 10⁻⁴ M by the fluorescence and its excitation spectra methods. At 10⁻⁴ M spectra coincide with the same spectra of corresponding dinucleotides. Revealed stacking aggregates do not dissociate at 85°C whereas the usual stacking aggregates in adenylic dinucleoside monophosphate dissociate at 27°C (data of UV and

CD spectroscopy). The excitation spectra of fluorescent stable-stacking aggregates have exciton splitting about 3500 cm⁻¹ that gives the unusually small interplane distance ~3.0 Å (dense packing). This small distance may be the cause of stability of aggregates. We suppose that the stability may also be caused by the water and cation bridges between the N atoms of the different molecules in the aggregate. These bridges play the role of sugar-phosphate chain in RNA molecules.

The stability of the fluorescent stacking aggregates of nucleic bases allows us to interpret them as precursors of RNA molecules in chemical evolution (proto-RNA).

The replication mechanism of stable stacking aggregates without any ferments based on consecutive rises and falls of temperature (1 - 65°C) is proposed. A new definition of life phenomenon is also proposed and the variants of life in systems without nucleic bases are discussed.

PI1**Two diastereomeric (6-4) adducts from photoreaction of uracil in frozen aqueous solution***M.D. Shetlar, V.J. Basus**School of Pharmacy, University of California, San Francisco, USA*

The photochemistry of uracil (Ura) has received much attention over the years, both when irradiated in liquid aqueous solution and in the frozen state. The characterized photoproducts in the liquid state include a hydrate and cyclobutane dimers; in ice, the main identified products are cyclobutane dimers and a dehydrated form of a putatively very unstable (6-4) adduct. During a re-examination of the photochemistry of uracil, we found that two diastereomeric (6-4) adducts are formed in unequal yields when Ura is irradiated in frozen aqueous solution at dry ice temperature. Both of the compounds have UV spectra similar in profile to those of known adducts of a similar nature; these spectra show λ_{max} at around 304 nm. Studies with nuclear magnetic resonance spectroscopy and mass spectrometry yielded data consistent with both of these compounds being (6-4) adducts, namely the *cis* and *trans* isomers of 5-hydroxy-6-4'-(pyrimidin-2'-one)-5,6-dihydrouracil. Finally, both of these compounds decompose upon heating or treatment with acid to form 6-4'-(pyrimidin-2'-one)uracil. The products probably arise from a common unstable precursor containing an oxetane ring, with the oxygen atom in the oxetane ring being attached at C5 of the component dihydrouracil moiety. In this proposed mechanism, attack of water at C5 of the uracil can occur on two different faces of the oxetane ring, such that resultant hydroxyl in the end product is either *cis* or *trans* to the pyrimidone ring.

Research support from the National Science Foundation during the course of this work is gratefully acknowledged.

PI2**Time-resolved study of thymine dimer formation***S. Marguet, D. Markovitsi**Laboratoire Francis Perrin CEA/DSM/DRECAM/SPAM - CNRS**URA 2453, CEA Saclay, 91191 Gif-sur-Yvette, France*

Absorption of UV radiation by DNA bases induces mutations which appear mainly at bipyrimidine sites. The major photoproducts are cyclobutane dimers and pyrimidine-(6-4)-pyrimidone adducts. Since the isolation of the first bipyrimidine dimers, large number of studies aiming at the understanding of the various factors which play a role in their formation have been published. In spite of the intense work in this field, there is still complete lack of information regarding the time-scales at which cyclobutane dimers and (6-4) adducts are formed. Laser flash photolysis experiments, which provide such information, are difficult to perform for DNA components. Indeed, data are distorted by excitation of accumulated photoproducts. Moreover, hydrated electrons and radical ions resulting from two photon ionization obscure absorption related to other processes occurring with low quantum yield.

In the present laser flash photolysis investigationⁱ, dedicated to the single stranded oligonucleotide (dT)₂₀, we managed to overcome the above difficulties. We show that direct excitation (266nm) of the oligonucleotide leads to cyclobutane dimers in less than 200 ns whereas the (6-4) adduct is formed within 4 ms via a reaction intermediate. The overall quantum yield for the (6-4) formation is $(3.7 \pm 0.3) \times 10^{-3}$ and that of the cyclobutane dimers $(2.8 \pm 0.2) \times 10^{-2}$. No triplet absorption is detected showing that either the intersystem crossing yield decreases by one order of magnitude upon oligomerization ($<1.4 \times 10^{-3}$) or the triplet state reacts with unit efficiency in less than 200 ns to yield cyclobutane dimers.

ⁱ S. Marguet, D. Markovitsi, *Time-resolved study of thymine dimer formation*, J. Am. Chem. Soc., 2005, 127, 5780

PI3**Photo-induced guanine radical cation in short oligonucleotide is able to cross-link with lysines containing peptide***S. Perrier¹, J. Hau², D. Gasparutto¹, J. Cadet¹, A. Favier¹, J.-L. Ravanat¹**¹DRFMC/SCIB/LAN, 17 rue des Martyrs CEA Grenoble, F-38054 Grenoble cedex 9, France; ²Nestlé Research Centre, Lausanne, Switzerland*

It is well known that the formation of radical within cellular DNA could induce the formation of DNA-protein cross-links. Guanine is the preferential DNA target of one-electron-mediated DNA oxidation and it has been shown that the guanine radical cation could be responsible for the formation of DNA-protein cross-links. As demonstrated with model systems, the guanine radical cation or derived radical could react with several nucleophiles such as water, methanol, 5'-hydroxyl group and amino groups to generate several modified nucleosides.

In the present work, the formation of DNA-protein cross-links involving the transient formation of a guanine radical cation was investigated using riboflavin-mediated photosensitization of TGT oligonucleotide in aerated aqueous solution in the presence of KKK tripeptide. Emphasis was placed on the identification of the structure and the determination of the mechanism of formation of the predominant oligonucleotide-peptide cross-links. Thus, two major TGT-KKK adducts were isolated by reversed-phase HPLC and characterized, as inferred from CD, NMR and mass spectrometry analyses. They were shown to be two diastereomers for which the ϵ -amino group of the central lysine of KKK is linked to the C8 position of the former guanine base both exhibiting a spirodiiminodihydroantoin structure. A detailed study of the mechanism of their formation allowed us to demonstrate that they both arise from the initial formation of a cross-link in which guanine is substituted at the C8 position by KKK. Interestingly, the latter adduct was found to be an efficient target to further one-electron oxidation, as observed for 8-oxodGuo, giving rise almost quantitatively to the two predominant diastereomeric adducts above mentioned.

Our work demonstrates that nucleophilic attack at the C8 position of the guanine radical cation by the amino group of a lysine could induce the formation of an adduct between an oligonucleotide and a peptide. Work is in progress in order to determine if other nucleophilic amino acids could also react at the C8 position of guanine radical cation, and attempts will be made to search for the formation of such DNA-protein cross-links in cellular DNA.

PI4**Crystal structure of the d(TpA) Thymine-Adenine photoadduct***R.J.H. Davies¹, J.F. Malone²**¹School of Biology and Biochemistry, ²School of Chemistry, Queen's University, Belfast, UK*

Direct UV excitation of the deoxydinucleoside monophosphate d(TpA) induces the formation of an intramolecular thymine-adenine photoadduct, denoted TA*. Although the quantum yield for production of TA* in native DNA is ~100-fold lower than for pyrimidine dimerization, there is evidence to suggest that TA* can act as a premutagenic lesion in both bacterial and human cells. From our initial characterization studies, we concluded that TA* was a cyclobutane derivative generated by [2+2] photoaddition of the 5,6 double bond of thymine across the C(6) and C(5) positions of adenine. Subsequently, however, Taylor and co-workers [Zhao *et al.* (1996) *Nucleic Acids Res.* 24, 1554] proposed that it was a less strained valence isomer of this structure containing an 8-membered heterocyclic ring. Their revised structure, assigned on the basis of extensive heteronuclear NMR measurements, is generally more compatible with the observed physicochemical properties of TA*. Here, we report a crystal structure analysis of TA* which confirms the molecular structure advanced by Zhao *et al.* and thus supports the view that TA* results from the isomerisation of a precursor cyclobutane photoadduct.

Evaporation of aqueous solutions of HPLC-purified TA* has been found to yield heavily hydrated microcrystals in the form of very thin plates. X-ray diffraction analysis was performed on a single crystal (0.35 x 0.06 x 0.06 mm) which allowed the connectivity of all non-hydrogen atoms in the photoadduct to be established unequivocally. Data were collected at 150 K to $2\theta = 57^\circ$ with MoK α radiation. 14148 reflections were measured, of which 2435 were unique and 1228 'observed' (i.e. $I > 2\sigma(I)$). All non-hydrogen atoms were successfully refined, with anisotropic vibration parameters, to $R1 = 9.0\%$. This revealed the presence of a central 1,3-diazocyclooctatriene ring as predicted by Zhao *et al.* However the positions of hydrogen atoms, and hence the tautomeric state, could not be determined.

PI5

Antigenotoxic activity of two essential oils (*Origanum compactum* and *Cinnamomum camphora*) against UV and 8-MOP+UVA induced mutagenic and recombinogenic events in diploid yeast (*Saccharomyces cerevisiae*)

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Essential oils and extracts obtained from many plants have recently gained popularity and scientific interest. Many essential oils are used as drugs in folk medicine and as ingredients in food, perfumes or cosmetics. In the present study, we have been interested in the evaluation of their possible antigenotoxic activities. We report here results obtained on the antigenotoxic effect of *Origanum compactum* and *Cinnamomum camphora* essential oils using ultraviolet C irradiation (UVC) and 8-methoxypsoralen (8-MOP) plus ultraviolet A (UVA) irradiation as photomutagens and diploid yeast (D7) as eukaryotic model system (*Saccharomyces cerevisiae*). UVC-irradiation produces mostly cis-syn cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts, whereas, 8-MOP+UVA induces monoadducts, interstand cross-links in DNA and oxidative damage. Our results show that addition of both essential oils at non toxic concentration during post-treatment incubation causes (1) a striking increase in the induction of cytoplasmic petite mutations and mitochondrial damage and (2) a significant decrease in the frequencies of point mutations (ILV⁺) and mitotic gene convertants (TRP⁺) induced at comparable survival levels. The results suggest that both essential oils may interfere with the expression of photo-induced mutations and mitotic intragenic recombination via the induction of mitochondrial damage and lethality (apoptosis).

PI6

Photochemistry of DNA in spores of *Bacillus subtilis*

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Spores are dormant forms of bacteria exhibiting increased resistance to a series of stress including UV radiation. UVC- and UVB-irradiation of spores leads to the formation of 5-thymine-5,6-dihydrothymine, the spore photoproduct (SP), as the sole DNA photoproduct instead of the cyclobutane dimers (CPD) and (6-4) photoproducts (64PP) generated between adjacent pyrimidines in other cell types. Using HPLC associated to tandem mass spectrometry, we individually quantified all these photoproducts in various strains of *Bacillus subtilis* and in isolated DNA in order to gain insights in the novel photochemistry of spore DNA. The specific formation of SP in wild type spores was first confirmed since no significant formation of CPDs and 64PPs could be observed. Furthermore, no evidence could be obtained for the

formation of a thymine-cytosine analog of SP. Then, the effect of α/β type small, acid-soluble proteins (SASP), which saturate the DNA in spores, was investigated. UVC-irradiation spores of mutant strains lacking SASP led to a lower yield of SP than in the wild type strain, concomitant with an increase in the yield of CPDs and 64PPs of the four possible bipyrimidine doublets. The ability of SASP to favor the formation of SP at the expense of CPDs and 64PPs was also observed in isolated DNA. Then, strains deficient in dipicolinic acid, a major component of the spore core, were exposed to UVC. Lack of DPA was found to make possible the formation of CPDs and 64PPs in significant yield and to decrease the yield of SP. Additional experiments showed that DPA is able to photosensitize isolated DNA and free thymidine, likely through a triplet energy transfer mechanism. Irradiation of spores of the double SASP/DPA mutant leads to a distribution of DNA photoproduct similar to that observed in vegetative cells, strongly suggesting that the combined effects of SASP and DPA are responsible for the specific formation of SP in spore.

PI7

Quantification of 4-hydroxy-2-nonenal adduct to glutathione and to DNA in humans cells

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Cellular components, in particular DNA, are target to a wide number of endogenous or exogenous genotoxics agents. Among them, Reactive Oxygen Species (ROS), produced under oxidative stress conditions, UV and γ irradiations, may damage DNA. The resulting lesions, including strand breaks and base damage have been extensively studied.

Recently, an indirect oxidative DNA damaging pathway, involving the final products of lipid peroxidation, has been proposed. Indeed, the decomposition of the cell membrane lipids by ROS, generates a series of aldehydes able to react with cellular components. In particular, they can diffuse into the cell's nucleus and alter DNA. However, cells have capacity to protect themselves against this degradation. Aldehyde's scavenging molecules are present in the cytosol, especially glutathione (GSH), the most important scavenger.

4-hydroxy-2-nonenal (4-HNE) is one of the most reactive aldehyde generated during the lipid peroxidation process. The conjugation reaction between glutathione and 4-HNE leads to an adduct formation, GS-HNE, limiting aldehyde's reactivity with other cellular components.

We study DNA damage caused by 4-HNE and the role played by glutathione to prevent from this degradation. THPI monocytes cells were treated by the aldehyde and reverse phase HPLC associated with a tandem mass spectrometer was applied, to the determination and quantification of alkylated DNA bases and GS-HNE.

This method allows the determination of the ratio between aldehyde conjugated by glutathione and aldehyde alkylating DNA bases. Such results will provide information on the biological relevance of the indirect ROS-mediated DNA damaging process.

PI8

Study of the photobiological effects of 8-methoxypsoralen (8-MOP) and UVA in eukaryotic cells using DNA microarray technology

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8-MOP plus UVA induced effects in eukaryotic cells involve a cascade of events underlying the cytotoxic, mutagenic and carcinogenic responses. Gene induction is one of the crucial initial events. Using genomic microarrays we report here transcriptional changes photoinduced in the 6300 genes of the yeast *Saccharomyces cerevisiae* following exposures to 8-MOP plus

UVA. This treatment induces DNA monoadducts and interstrand cross-links as well as oxidative damage involved in genotoxic effects but also in beneficial photochemotherapeutic effects.

The repair of 8-MOP photolesions implies the formation of DNA double-strands breaks (DSB) as intermediates, the activation of the homologous recombination pathway (involving the *RAD51* gene) and a signal transduction cascade (including the kinases Mec1/Rad53, equivalent to ATR in human cells). A total of 176 genes responded to 8-MOP plus UVA treatment. Of these, 140 and 36 genes, respectively, showed a significant increase and decrease in expression. Changes in gene expression encompassed a wide variety of functions including DNA and general metabolism, stress responses and transport. Survival of deletion mutants was measured as well. Many DNA damage inducible genes like *RNR1*, *RNR2*, *RNR3*, *RNR4*, *DIN7*, *RAD16*, *RAD51*, *RAD54*, *DUN1* were induced by 8-MOP plus UVA. Some of them were previously recognized as specifically DNA damage responsive genes.

Our results suggest that 8-MOP plus UVA induces specific transcriptional profiles showing a general signature of DNA damage but also reflecting the specific types of photodamage induced.

PI9

Comparison between 8-oxo-7,8-dihydro-2'-deoxyguanosine and 1,N²-etheno-2'-deoxyguanosine levels in lung DNA of rats exposed to cigarette smoke

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Cellular damage caused by reactive oxygen and nitrogen species has been proposed to be an important factor in human pathologies. These species are produced in cells through a series of processes, including oxidative metabolism, and exposure to physical agents such as UV light and ionizing radiation. Several studies have provided information that allow the use of the modified bases as markers of oxidative stress. We investigated here the levels of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) and 1,N²-etheno-2'-deoxyguanosine (etheno-dGuo) in lung DNA of rats exposed to cigarette smoke. As the main noteworthy result, rats exposed to cigarette smoke for 180 days showed significantly increased levels of 1,N²-etheno-2'-deoxyguanosine compared to control. However, no changes in the levels of 8-oxodGuo were observed. The data suggest that the induction of etheno-dGuo adduct in DNA may be a useful marker of DNA damage. On the other hand, it is well known that 8-oxodGuo is highly susceptible to various oxidizing agents oxidation reactions. It will now be important to assess if the products of 8-oxodGuo oxidation are formed at the cellular level upon exposure to different oxidizing agents.

Acknowledgements: Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP (Brazil), the Conselho Nacional para o Desenvolvimento Científico e Tecnológico, CNPq (Brazil), Pró-Reitoria de Pesquisa da Universidade de São Paulo (Brazil). P.D.M. is the recipient of a fellowship from the Guggenheim Foundation.

PI10

Reaction of singlet oxygen with 2'-deoxyguanosine and 8-methoxy-2'-deoxyguanosine

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Many key biomolecules such as DNA, proteins and unsaturated lipids are potential targets for oxidation reactions mediated by

singlet molecular oxygen (¹O₂). It is well documented that guanine is among nucleic acids components the exclusive target towards ¹O₂ oxidation. Studies conducted with free 2'-deoxyguanosine (dGuo) in aqueous solution have led to the identification of two main oxidation products that consist of a pair of diastereomeric spiroiminodihydantoin nucleosides (dSp) together with smaller amounts of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo). However, the mechanism of their formation has not been fully clarified yet. The present work provides additional mechanistic information on the formation of the main ¹O₂-oxidation products of dGuo and a related model, namely 8-methoxy-2'-deoxyguanosine (8-MeOdGuo). This was achieved using the thermolysis of a naphthalene derivative endoperoxide as a clean source of ¹⁸O-labeled ¹O₂ and by performing additional labeling experiments with H₂¹⁸O. The products thus formed, were detected and quantified using the accurate HPLC-ESI-MS/MS method. We observed that dGuo oxidation by ¹O₂ leads to the initial formation of 8-oxodGuo followed by its consumption; in addition the H₂¹⁸O labeling experiment showed, in fact, that the secondary oxidation pathway might be significant in the formation of dSp. Furthermore, in order to gain further insights into the ¹O₂-mediated degradation pathways of guanine derivatives, we used as a suitable model, the modified nucleoside 8-MeOdGuo that mimics the 8-enolic tautomeric conformation of 8-oxodGuo. Interestingly, the reaction of ¹O₂ with 8-MeOdGuo gives rise to similar oxidized nucleoside products that those obtained for dGuo and 8-oxodGuo oxidation. The results thus obtained, contribute to a better understanding of the mechanistic aspects of the ¹O₂ mediated oxidation reactions involving guanine derivatives.

Financial support: FAPESP, CNPq, PRONEX and Guggenheim Foundation (P.D.M.).

PI11

Steady-state and time-resolved studies on Ketoprofen-thymine dyads

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A number of drugs are capable of producing photosensitizing effects, such as light-induced abnormal skin reactions, which are often classified as phototoxic. These reactions appear when certain skin areas of treated patients are exposed to sunlight.

In recent years, Ketoprofen (KP) and related non-steroidal anti-inflammatory agents including Benoxaprofen, Carprofen, Naproxen and Tiaprofenic Acid have been reported to exhibit photosensitizing properties. The involved molecular mechanisms have been studied in some detail, but a fully consistent picture is still lacking.

In particular, KP produces DNA damage *via* oxidative processes, as well as energy transfer leading to thymine dimers formation. In the present work, synthetic nucleosides have been prepared by tethering a KP unit to thymidine nucleoside at different positions of the sugar.

Steady-state irradiation of the obtained dyads leads to a wide variety of photoproducts (different regio and stereoisomeric oxetanes, as well as the products resulting from intramolecular coupling of the biradicals formed after hydrogen abstraction from the thymine methyl group and/or the activated sugar positions).

Time-resolved experiments have allowed to quantify the reactivity of the benzophenone-like excited triplet state, which depends on the position of the KP attachment and on the length of the spacer.

Competition between oxetane formation and intramolecular hydrogen abstraction is discussed in connection with the DNA photosensitization mediated by KP.

PI12**Photoinduced binding of ruthenium trisbipyrazine on 8-oxoguanine and inhibition of the replicative T4 polymerase***N. El-Akra, J.-P. Souchard, P. Vicendo**Laboratoire des IMRCP, UMR 5623 au CNR, Université Paul Sabatier, 118 route de Narbonne 31062 Toulouse Cedex, France*

Trisbipyrazine ruthenium(II) complex is known to form covalent photoinduced linkages with DNA via an electron transfer process. In order to determine the sequence selectivity of this chemistry "foot printing experiments", using the DNase I, were performed. Polyacrylamide sequencing gel revealed "hot spots" which occurred at adjacent, guanines (GG, GGG), with a particularly strong preference for GGG sites. These data are consistent with our previously postulated mechanism for the covalent binding chemistry which involves an electron transfer from the deoxyguanine to Ru(bpz)₃²⁺ at the excited state. To get further insight on the biological relevance of DNA photo-adducts of Ru(bpz)₃²⁺, *in vitro* replication studies were performed. After illumination, polymerase action is inhibited at multiple sites in the vicinity of the dye lesions corresponding to GG and GGG sites. These observations support a model in which Ru(bpz)₃²⁺ reacts with guanine responsible of potential gaps in DNA and can in this way impede primer elongation by T4 DNA polymerase. This assumption was supported by original data showing a covalent binding of Ru(bpz)₃²⁺ on the 8-oxoguanine, which has a lower redox potential than the guanine, and a selective stop of the DNA polymerase T4 action at this site. For this study a single 8-oxoguanine was introduced in the sequence of the proto-oncogene H-ras. All these results open new perspectives for future application of Ru(bpz)₃²⁺ as a new drug for the photodynamic therapy.

PI13**Photostability of ruthenium complexes in presence of proteins: influence in DNA photosensitization***L. Bijeire, P. Vicendo**Laboratoire des IMRCP, UMR 5623 au CNRS, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse Cedex, France*

Polypyridyl ruthenium complexes are very attractive compounds in the area of DNA photochemistry. They offer a large panel of biochemical applications such as chemical photonucleases, DNA chiral probes, DNA alkylating agents. Their photoreactivity with nucleic acids partly stems from the redox potential of their metal-to-ligand charge transfer excited state. In previous work we have reported that Cu/Zn superoxide dismutase (SOD) induced an unexpected enhancement of the photonuclease activity of both Ru(bipy)₃²⁺. DNA photosensitization experiments in the presence of deferoxamin and catalase clearly show that this augmentative effect is not due to the involvement of superoxide anion and hydroxyl radical. Moreover, in the presence of SOD a dramatic increase of the photodecomposition of Ru(bipy)₃²⁺ is observed. This effect, leading to the formation of RuL₂LO and RuL₂LO₂, is due to an attack of Ru(bipy)₃²⁺ by singlet oxygen which is trapped in close proximity to the metal complex. This cage effect is attributed to the SOD which creates a constraining cage around the complex. This is in accordance with EPR experiments using TEMP showing a decrease of singlet oxygen production when Ru(bipy)₃²⁺ is irradiated in the presence of SOD. In an attempt to get further mechanistic informations, oligonucleotide sequencing experiments were carried out. Alkali labile sites were revealed by a piperidine treatment. In the absence of SOD, all guanines were oxidized without sequence selectivity as it was expected with a singlet oxygen attack. In contrast, in the presence of SOD, guanines in 5' of a GG site were selectively oxidized, signature of an electron transfer process. This reactivity may be attributed to the species RuL₂LO and RuL₂LO₂.

PI14**Spore photoproduct lyase -characterization of an iron-sulfur DNA repair enzyme***O. Berteau¹, S. Ollagnier-de-Choudens¹, T. Douki², M. Atta¹, M. Fontecave¹**¹Laboratoire de Chimie et Biochimie des Centres Rédox Biologiques, DRDC-CB, Unité Mixte de Recherche 5047 Commissariat à l'Energie Atomique / CNRS / Université Joseph Fourier, Grenoble, France; ²Laboratoire Lésions des Acides Nucléiques, DRFMC/SCIB UMR-E 3 CEA-UJF, CEA-Grenoble, France*

The DNA of all organisms is subject to modifications under exposure to cytotoxic and mutagenic agents. It is also well known that genotoxic photoproducts such as cyclobutane pyrimidine dimers (CPDs) and (6-4) adducts formed by cycloaddition of two adjacent pyrimidine bases are generated in DNA when cells are exposed to UV light. In contrast, under certain conditions, such as within bacterial spores, irradiation of DNA gives rise to the unique spore photoproduct lesion (SP) (5-thymine-5,6-dihydrothymine). This peculiar photochemical reactivity is currently explained by the high degree of dehydration occurring in dormant spores as well as unique packaging conditions.

The bacterial spores contain a specific DNA repair enzyme, named Spore Photoproduct Lyase (SP Lyase) to repair the SP lesion. It is now well established that SP Lyase is a member of the "radical-SAM" family. Indeed, preliminary spectroscopic and biochemical studies have shown that: (i) the protein carries an [4Fe-4S] cluster, (ii) the reaction is absolutely dependent on S-adenosylmethionine (SAM); (iii) the repair mechanism involves a 5'-deoxyadenosyl radical (Ado°) generated from reductive cleavage of AdoMet.

The SP Lyase provides a novel aspect of the diversity of DNA repair mechanisms in living organisms. It is thus worth further investigating the structure of this enzyme, its substrate specificity and its mechanism. To date only limited biochemical information is available, probably due to the inherent instability of the protein. Here, we report an additional characterization of a highly pure preparation of recombinant SP Lyase from *Bacillus subtilis*. In particular we provide a detailed spectroscopic characterization of the cluster and we show that the SP lesion under the form of a dinucleoside monophosphate (SPTpT) is recognized with high specificity by the prepared enzyme, and that the fourth cysteine, Cys 141, present in the sequence plays an important role in the repair reaction.

PI15**DNA-DNA crosslinking at guanines: a new and major class of oxidatively generated damage by UV laser biphotonic ionization in DNA***H. Menoni^{1,2}, D. Gasparutto³, J.-L. Ravanat³, J. Cader³, S. Dimitrov⁴, D. Angelov^{1,2}**¹Ecole Normale Supérieure de Lyon, Laboratoire Joliot-Curie/UMR 5161, 46, allée d'Italie, 69007 Lyon, France; ²Institute of Solid State Physics, Bulg. Acad. Sci., 72, Tsarigradsko Shaussee Blvd., 1784 Sofia, Bulgaria; ³Laboratoire Lésions des Acides Nucléiques, SCIB-UMR-E n°3 CEA-UJF, DRFMC CEA/Grenoble, 38054 Grenoble, cedex, France; ⁴Institut Albert Bonniot, INSERM U309, Domaine de la Merci, 38706 La Tronche, Cedex, France*

Exposure of DNA to high intensity UV laser pulses results in the induction of specific oxidized bases, which arise via biphotonic ionization of nucleobases. The photodamage formation depends on both the DNA sequence and the structure of the biopolymer. DNA oxidative lesions, generated by either UV laser biphotonic ionization or type I photosensitization are localized predominantly at guanine sites in a manner that is sequence and structure dependent. This is mostly due to hole migration and trapping of the initially generated radical cations by guanines that exhibit the lowest ionization potential among DNA components. The guanine radical cation is rapidly transformed through competitive deprotonation or water addition steps to the oxazolone nucleoside and 8-oxo7,8-dihydro-2'-deoxyguanosine respectively. The *N*-

glycosidic bond of the two latter lesions is cleaved by alkali treatment and upon incubation with the formamidopyrimidine DNA N-glycosylase (Fpg protein) respectively. This enables their mapping at a nucleotide level using the appropriate treatment and sequencing gel electrophoresis analysis.

In this work, we report the UV laser induction of a new predominant DNA oxidatively generated lesion, namely DNA-DNA interstrand covalent adducts. A series of experiments on defined sequence oligonucleotides allowed demonstrating the biphotonic ionization nature of the photoprocess that involves the guanine radical cation as the precursor of DNA-DNA adducts. Chemical analysis of the structure of the cross-links by means of HPLC-MS/MS is under way.

The occurrence of this modification is highly sensitive to structural deformations induced by specific protein binding, especially to local DNA base pair opening. This has been checked on the four-way junction (4WJ)-Ruv A complexes. Design of functional 4WJ-RuvA,B,C complex aimed at real time branch migration dynamics study is currently in progress.

Acknowledgements: this work was supported by the European Commission through network grant *MCRTN-CT-2003-505086 (CLUSTOXDNA)*.

PI16

Ozone induces singlet molecular oxygen generation in the presence of DNA

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Ozone (O₃) is one of the major toxic compounds present in the polluted urban atmosphere. Recently attention has been focused on the possibility of endogenous O₃ production by antibodies and this has been a matter of extensive debates. The toxicity of O₃ appears to be due to its ability to promote oxidative damages to important biomolecules, such as, lipids, proteins and DNA, and particularly due to its mutagenic potential. Treatment of DNA with O₃ has been shown to cause strand-breaks, base oxidations and adducts formation. However, the mechanisms involved in O₃ genotoxicity still remains largely unknown. It has been postulated that O₃ can exert its genotoxic effects by direct reaction with the target molecules, such as dG, or by production of secondary reactive oxygen species, such as, hydroxyl radicals, hydroperoxides, and singlet molecular oxygen (¹O₂). Here, we investigated the possibility of ¹O₂ production during interaction of O₃ with the DNA bases (dG, dA, dC and dT) and calf thymus DNA. The generation of ¹O₂ was monitored by the measurement of light emission at 1270 nm using a photomultiplier coupled to a monochromator. When a solution containing DNA nucleosides or deoxyribose (1 mM in D₂O) was bubbled with O₃ gas or was mixed with an O₃ saturated solution containing 0.1 mM of O₃, an intensive production of ¹O₂ was observed exclusively with dG and dA. Similar result was also obtained by bubbling O₃ into a solution containing calf thymus DNA. These results indicate that O₃ interacts particularly with dG and dA generating ¹O₂ and this may contribute to the genotoxicity mediated by O₃. Supported by: FAPESP, CNPq, USP e Guggenheim Foundation (P.D.M.).

PI17

Interplay of nitration and oxidation reactions in DNA: Insights from laser flash photolysis and oxygen-18 labeling experiments

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In living tissues under inflammatory conditions, reactive oxygen and nitrogen species are known to cause the oxidation and nitration of cellular DNA. However, the mechanisms of action are poorly understood. The oxidation and nitration reactions in DNA

associated with the combination of nitrogen dioxide radicals with 8-oxo-7,8-dihydroguanine (8-oxoGua) and guanine radicals have been explored by kinetic laser spectroscopy and mass-spectrometry methods. In the case of 8-oxoGua, the major end-products of this bimolecular radical-radical addition are spiroiminodihydroantoin lesions, the products of 8-oxoGua oxidation. Oxygen-18 isotope labeling experiments reveal that the O-atom in the spiroiminodihydroantoin lesion originates from water molecules, not from nitrogen dioxide radicals. In contrast, the combination of nitrogen dioxide and guanine neutral radicals generated under the same conditions results in the formation of the nitro products, 5-guanidino-4-nitroimidazole and 8-nitroguanine adducts. The mechanistic aspects of the oxidation/nitration processes and their biological implications are discussed. Supported by NIH Grant 5 R01 ES11589.

PI18

Miniaturized instrumentation for the high throughput screening of DNA repair inhibitors

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We are developing a linkage between the acrylamide gel electrophoresis assay for nucleotide excision repair (NER) and the microfabricated capillary array electrophoresis microplates and detection system that has been developed for DNA sequencing by Professor Richard Mathies at the University of California, Berkeley¹. NER is a DNA repair pathway that removes bulky adducts which are produced by physical and chemical carcinogens, including the critical cytotoxic lesions formed by the cancer therapeutic Cisplatin. Although Cisplatin is a valuable agent in the treatment of various solid carcinomas, its clinical efficacy is limited by the development of resistant tumor cells. There is a large body of evidence that NER is an important source of resistance to platinum compounds. Such evidence includes cell culture studies showing that: 1) cells deficient in NER proteins have increased Cisplatin sensitivity (versus WT); 2) NER proteins and repair activity are increased in tumor resistant cell lines; 3) Cisplatin sensitivity and resistance correlate with mRNA levels of NER proteins in clinical specimens; and 4) Cisplatin resistant cell lines show that increased repair of Cisplatin DNA adducts facilitates tumor resistance. The overall goal is the development of a complete, low volume, low cost, high sensitivity assay for NER and for inhibitors of NER; this is an essential step in the discovery of a small molecule selective inhibitor of the NER pathway which, when administered in combination with Cisplatin, will enhance the cytotoxicity of platinum-based chemotherapeutics.

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PI19

Effect of (5'S)-5',8-Cyclo-2'-deoxyadenosine on the conformation of di and trinucleotides

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The 5S and 5R diastereomers of 5',8-cyclo-2'-deoxyadenosine (cdA)ⁱ are generated by •OH-mediated hydrogen atom abstraction at C5' of 2'-deoxyadenosine and subsequent intramolecular cyclization. These tandem lesions have been postulated to be key bulky DNA damageⁱⁱ implicated in the neurological side-effects that are observed in xeroderma patients who suffer for a deficiency in nucleotide excision repair (NER) pathwayⁱⁱⁱ. In order to delineate the conformational changes induced by the presence of cdA in DNA we have investigated the structures of 5'S

diastereomer of cdA, TpcdA, and TpcdApT by means of NMR spectroscopy and density function theory (DFT). Phosphorus-decoupled ^1H NMR experiments have been applied to determine the vicinal proton-proton coupling constants whereas the 2D NOESY technique has been used for the determination of the proton-proton distances. The NMR results have been compared with the data derived from the DFT calculations. The obtained results unequivocally demonstrate that cdA constitutes the stable element of the structure which is quite rigid. Moreover only slight structural changes have been observed for the di and trinucleotide in the temperature range 25-41°C. It can be postulated that the rigid structure of cdA would significantly influence the global structure of DNA. Work is in progress to further assess the conformation changes induced by either of the diastereomeric cdA in double-stranded DNA fragments. This should provide some explanation at the molecular level for the observed differences in the processing of cdA diastereomers by NER enzymes and translesional synthesis proteins.

Acknowledgment: this work has been partly supported by a EU grant, project "CLUSTOXDNA" MCRTN 505586.

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PI20

Study of 8-bromo-2'-deoxyinosine by photochemical and radiolytic methods

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When DNA is exposed to ionising radiation, decomposition products such as 5',8-cyclopurines are formed. From a mechanistic point of view, the C5' radical initially generated by hydrogen abstraction, intramolecularly attacks the aromatic ring of the base to form cyclopurines as the final oxidation products.

Recently, 8-bromo-2'-deoxyadenosine (**1**) and 8-bromo-2'-deoxyguanosine (**2**) have been studied by photochemical and radiolytic methods. It was found that **1** produces the 2'-deoxyadenosin-5'-yl radical; its cyclisation generates the corresponding aminyl radical (precursor of cyclopurines). On the other hand, **2** captures one electron forming the radical anion that undergoes protonation at C8 to afford 2'-deoxyguanosine.

Following with 8-bromo-2'-deoxypurines, the aim of the present work was to synthesize a new halodeoxypurine, namely **8-bromo-2'-deoxyinosine**, and to study the generation and reactivity of the 2'-deoxyinosin-5'-yl radical **R1**. For this purpose, both pulsed and continuous radiolysis, as well as steady-state and time resolved photolysis have been used.

At the transient level, formation of the cyclised aminyl radical was observed from **R1**, and its reactivity was analysed towards $\text{Fe}(\text{CN})_6^{3-}$, methylviologen and molecular oxygen. Furthermore, studying final products of steady-state photolysis and continuous radiolysis formation of the two 5',8-cyclo-2'-deoxyinosine isomers together with the 5'-carboxaldehyde-2'-deoxyinosine has been observed.

PI21

A plasmid biochip for DNA repair measurement, adaptation of HeLa cells to UVB irradiation

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It is well known that Nucleotide Excision Repair (NER) is the repair mechanism that process DNA photoproducts. The NER repair pathway is based on four sequential steps: recognition of the lesion, incision, excision, and a repair synthesis. Measuring NER repair activities is very important for understanding cell NER regulation, and cell NER adaptation to Ultra-Violet (UV) wavelength irradiation.

Wood *et al* who designed the very first *in vitro* cell free assay allowing measurement of NER activities. This assay takes advantage of the NER mechanism to incorporate radio-labeled nucleotides during the repair synthesis step of damaged plasmid by cell extracts. This very smart assay has two drawbacks, first it uses radioactivity and secondly it is time consuming. Based on this knowledge, we have designed a miniaturized *in vitro* non radioactive cell free assay using the same principle as the Wood *et al*'s one, for measuring NER and BER long patch repair activities. Thanks to the microarray format, this assay gives access to parallel measurement of repair activities of different DNA damage. We thus obtain DNA repair activities profiles for the tested cell types.

In a first approach, we have validated our assay using : polymerase inhibitors, complementation test with *Xeroderma pigmentosum* cells. We have determined repair profile of different cell lines : HeLa, Fibroblasts, Keratinocytes, Neurons. Now we focus on adaptation of HeLa cells to UVB. We showed that a moderate UVB dose (0,005 J/cm²) does not affect repair activities measured 24h and 48h after exposure. On the other hand a high UVB dose (0,025 J/cm²) globally strongly reduced repair activity at 24h. Repair activity was partly recovered 48h after exposure. Finally if an adaptive experiment is performed (0,005J/cm² then 0,025J/cm² at 24h) repair activity is globally increased 48h after first exposure. This shows that a moderate UVB dose prepares cells to higher dose.

PI22

Formation and biological significance of one-electron oxidation products of 5-hydroxypyrimidine residues

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Oxidatively generated damage to DNA arises continuously in cells and is likely to be involved in aging processes and several human pathologies, such as cancer and neurodegenerative diseases. This genetic alteration may be induced by endogenously generated reactive oxygen species during aerobic cellular metabolism. Other oxidation processes, which are of exogenous origin, include ionizing radiations, UVA-mediated photosensitization and various chemical agents. Thus, several reactive oxygen and reactive nitrogen species, radicals and one-electron oxidants such as photosensitizers (flavines, menadione,...) give rise to reactions with DNA commonly resulting in base modifications (more than 50 oxidized nucleosides have been identified at this date). Among the four DNA nucleobases, guanine, that exhibits the lowest redox potential ($E=1.29$ V), is the most susceptible to one-electronoxidation, and one of its main oxidized compounds, namely 8-oxo-7,8-dihydroguanine (8-oxoGua), has been extensively studied in terms of formation, repair, and mutagenicity. Moreover, the latter modified purine base ($E=0.58$) is readily subjected to further oxidation reactions which have recently become a matter of interest.

Emphasis was placed in this work on the formation, the reactivity and the biological impact, in term of enzymatic repair and replication, of several one-electron oxidation products of 5-hydroxyuracil (5-ohUra - $E=0.64$). Thus, the reactivity toward chemical and photochemical oxidative processes of the latter 5-hydroxypyrimidine residue has been investigated. Data will be provided regarding the structure, the stability and the features toward DNA repair enzymes of the overoxidized base lesions formed, namely dialuric acid and isodialuric acid.

PI23

Modification of the genotoxic effect of UV-irradiation by chemicals

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The development of a skin cancer is multistage process, in which the effect of environmental factors on DNA is crucial. Several methods are currently used to measure DNA-damage. The comet-assay and micronucleus-assay show different types of DNA-damage at different time points after the injury. A very sensitive biological end point for DNA damage is the measurement of DNA single-strand breaks. It can be studied at single cell level by means of comet-assay (single cell gel electrophoresis). The induction of single-strand breaks can also be the result of nucleotide excision repair, therefore the assay shows DNA repair capacity as well. Micronuclei represent genetic material that is not incorporated into the daughter nuclei at the time of cell division. The measurement of the induction of micronuclei is a very sensitive parameter for cytogenetic (clastogenic and aneugenic) damage. In the present study we demonstrate the DNA-damaging effect of UVB- and UVA-irradiation in different skin cell types and the modifying effect of a therapeutic photosensitizer (8-MOP) and an important air pollutant (formaldehyde) on UV-induced DNA-damage. Irradiation of cell cultures caused wavelength dependent, time-specific changes in DNA-migration in alkaline comet-assay. 8-MOP-treatment prior to UVA resulted in DNA-lesions detectable by neutral comet-assay. Formaldehyde causes preferentially DNA-protein crosslinks. We have found that formaldehyde interfered with the nucleotide excision repair induced by UV. As a consequence an enhanced UV-induced chromosomal damage could be observed in fibroblasts treated by formaldehyde prior to irradiation as measured by micronucleus-assay. It could be clearly demonstrated that parallel investigation of different biological endpoints for DNA-damage might lead us to a better understanding of the mechanism of photocarcinogenesis and photococarcinogenesis.

PI24

Studies on the photocleavage of nucleic acids by the dibenzo[b,g]quinolizinium salt and its dimer

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The design of drugs that interact with DNA and which may be used for its detection or deliberate degradation is an important goal in photobiology. Especially cationic dyes have been shown to bind efficiently to DNA and to induce photocleavage¹. Among the cationic aromatic compounds that have been investigated along these lines are benzo-annelated quinolizinium salts such as coralyne, a well-known protoberberine derivative, and derivatives thereof². In order to understand the parameters that govern the DNA-binding properties of annelated quinolizinium salts, attempts have been made to synthesize and study the linearly annelated analogue of protoberberine, namely the dibenzo[b,g]quinolizinium ion; however, the investigation of this compound suffers from its

rapid and selective dimerization in solution to the formal [4+4] cycloaddition product. In this contribution, studies on the DNAbinding and the DNA-damaging properties of the dibenzo[b,g]quinolizinium ion and its dimer will be presented. The monomer binds to DNA with a binding constant of $K = 1.3 \times 10^5 \text{ M}^{-1}$, whereas the dimer associates to DNA rather weak, presumably by electrostatic interaction. Notably, the irradiation of DNA in the presence of the dimer under anaerobic conditions leads to more strand breaks than in the presence of the monomer. Investigations with isopropanol and *t*-butanol as radical scavengers suggest a radical mechanism for the DNA damage.

Mechanistic details of this photoreaction will be presented.

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PI25

Long-time fluorescence components of poly(dA).poly(dT)

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The singlet excited states of monomeric DNA components (nucleosides, nucleotides) decay all at the femtosecond timescale. This ultrafast relaxation has been invoked to explain the DNA stability towards damage provoked by UV radiation. However, photoreactions do take place but their relationship to the excited states remains unknown. In the case of bichromophoric reactions, such as bipyrimidine dimer formation, the chromophores have to adopt appropriate conformations during the excited state lifetime. Thus, it is expected that the reaction probability increases with increasing lifetime.

Several literature data report fluorescence decays of hundreds of picoseconds or nanoseconds for DNA single and double helices. In particular, long time components have been reported for the single helices (dT)_n and double helices (dA)_n(dT)_n whose steady-state spectra do not show "excimer like" emission. We have shown that these decays are contaminated by the fluorescence of photoproducts. This is understandable if we consider that the orders of magnitude of DNA fluorescence quantum yield, the yield for the (6-4) dimer formation and the fluorescence quantum yield of (6-4) dimer are, respectively, 10⁻⁴, 10⁻³ and 10⁻². We have overcome the above difficulties by performing time-correlated single photon counting experiments in which the laser intensity (100 fs; 267 nm) at the sample surface was kept low enough to yield identical decays in successive measurements.

PI26

A comparison of solar UV induced DNA-damaging effects between Southern and Central Europe and Arctic high latitudes

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The stratospheric ozone concentration has been investigated by several methods, e.g., determinations of the ozone layer using a network of ground based spectrophotometers, of the Dobson and the Brewer types. These data indicate significant decrease of the ozone layer superimposed by much larger seasonal changes at specific geographical locations. The stratospheric ozone plays an important role in the attenuation of the short-wavelength components of the solar spectrum, thus the consequence of the

decreased ozone layer is an increased UVB level. Various pyranometers measuring the biological effect of environmental UV radiation have been constructed with spectral sensitivities close to the erythema action spectrum defined by the CIE. Using these erythemally weighted broad-band instruments to detect the tendency of UVB radiation controversial data have been found. To quantify the biological risk due to environmental UV radiation it is reasonable to weight the solar spectrum by the spectral sensitivity of the DNA damage taking into account the high DNA-sensitivity at the short wavelength range of the solar spectrum. Various solar UV sensitive biological dosimeters have been developed e.g., polycrystalline uracil thin layer. These are usually simple biological systems or components of them. Their UV sensitivity is a consequence of the DNA-damage. We show that biological dosimeters applied for long-term solar UV monitoring are promising tools for the assessment of the biological hazard. For comparing solar UV induced DNA-damage between high latitudes in Arctic regions with observations in central and southern Europe we started during 2004 measurement campaigns at facilities of the Polar Geophysical Institute of the Russian Academy of Sciences in Barentsburg/Spitsbergen (78 degrees north) and Apatity (68 degrees north). We will present the first preliminary results of these field experiments and discuss planned future experiments, which will be carried out during 2005.

PI27

Ground-based measurement of solar light intensity (illuminance), temperature, and relative humidity from the tropics (Turen, East Java, Indonesia) between 1995 and 2001

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Long-term measurement of illuminance on ground level, temperature, and relative humidity (RH) was conducted in Turen, East Java, Indonesia (7.5°S, 112.8°E, 300 m a.s.l.) from December 1995 until August 1997, June to October 2000, and February to June 2001. The data were collected three-times a day, representing morning (8 am), noon (12 am), and afternoon (4 pm), respectively. Daytime in the region is about 12 hours ranging from about 5:30 in the morning until about 5:30 in the afternoon, almost unchanged over the year. Comparing monthly average data from December 1995 throughout June 2001, we observed a significantly (ANOVA, $P < 0.001$) higher illuminance during dry season (DS, April to September) 1996-1997, in comparison with the data in DS 2000-2001. The difference between both periods was more pronounced in the afternoon data. The averages of illuminance were 51,164 lm/m² (8 am), 88,565 lm/m² (noon), and 38,440 lm/m² (4 pm) during DS 1996-1997, and 31,342 lm/m² (8 am), 69,847 lm/m² (noon), and 5,570 lm/m² (4 pm) during DS 2000-2001. No significant differences were observed between rainy season (RS, October to March) 1996-1997 and RS 2000-2001 for the morning and noon data. However, a significantly higher illuminance ($P < 0.001$) was observed in the afternoon during RS 1996-1997 (16,746 lm/m²) than in RS 2000-2001 (6,130 lm/m²).

Temperatures during DS 1996-1997 in the morning (28.1°C) and at noon (32.3°C) were significantly lower ($P < 0.001$) compared to DS 2000-2001 (29.2°C at 8 am, and 34.8°C at noon), however, in the afternoon the opposite was observed (29.2°C for DS 1996-1997 and 28.7°C for DS 2000-2001) ($P < 0.01$). No significant differences of temperature were observed in the morning and afternoon between RS 1996-1997 and RS 2000-2001. At noon however, a significantly lower ($P < 0.05$) temperature was observed during RS 1996-1997 (33.6°C) compared to RS 2000-2001 (34.7°C).

No significant differences of RH were observed in the morning between DS 1996-1997 and DS 2000-2001, and between RS 1996-1997 and RS 2000-2001. However, significantly ($P < 0.001$) higher RH was observed for noon and afternoon data of DS 1996-1997 (57.6 % at noon and 58.5 % at 4 pm) compared to DS 2000-2001

(51.2 % at noon and 56.0 % at 4 pm), and of RS 1996-1997 (60.8 % at noon and 63.1 % at 4 pm) compared to RS 2000-2001 (52.8 % at noon and 58.9 % at 4 pm).

The anomalies during 1996 and 1997 might be related to the occurrence of an unusually strong El Niño event during that period.

PI28

Effects of sublethal (7.4 W/m²) UVA irradiation on activity levels of oxidative defense enzymes (catalases, superoxide dismutases, glutathione reductase) and on protein oxidation and survival after lethal UVA in *Escherichia coli*

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Previous research suggests that UVA radiation (400-315 nm) mediates its biological effects on bacteria via reactive oxygen species (ROS), including the superoxide radical (O₂⁻), hydroxyl radical (OH[•]), hydrogen peroxide (H₂O₂), and singlet oxygen (¹O₂). *E. coli* has evolved antioxidant defense mechanisms to protect against the accumulation of ROS. Continuous sublethal UVA irradiation administered to *E. coli* cells at a very low fluence rate (7.4 W/m²) leads to an adaptive response, changing the activity levels of hydroperoxidases (HPI, HPII), glutathione reductase (GR) manganese superoxide dismutase (MnSOD) and iron superoxide dismutase (FeSOD). It also attenuates the growth-delay response and increases resistance to lethal UVA irradiation. However, this adaptive response induced by sublethal UVA irradiation does not protect wild type *E. coli* cells from protein damage after challenge with lethal UVA irradiation. When *E. coli* is exposed to 45 min of UVA irradiation (total dosage 135 kJ/m²) delivered at a fluence rate (50W/m²), extensive protein oxidation (carbonyl groups) occurs. Reduced protein oxidation, as measured by carbonyl groups, in mutant strains lacking SOD and Fur (ferric uptake regulator), suggests that in these strains, other targets of oxidative damage or other types of protein oxidation contribute to cell lethality due to a shift in balance between prooxidant and antioxidant activities.

PI29

The role of Bach-1 and NF-E2-related factor 2 in ultraviolet A (oxidant) mediated activation of heme oxygenase-1 in human skin cells

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Up-regulation of heme oxygenase 1 (HO-1) by ultraviolet A (UVA) (320 - 380 nm) radiation provides a crucial cellular defence mechanism against oxidative stress in human skin but the events underlying HO-1 regulation by UVA remain poorly characterised in human cells. In murine models, work with other inducers (heavy metals, heme) has demonstrated that HO-1 is transcriptionally activated by NF-E2- Related Factor 2 (Nrf2) or repressed by Bach-1 binding at MARE (Maf Recognition Element) sites. The activities of both Bach-1 (a heme-binding protein) and Nrf2 appear to be influenced by levels of free heme since Bach-1 bound to heme does not bind to the MARE site and heme stabilises Nrf2 protein levels. We now provide evidence for the involvement of both Nrf2 and Bach-1 in UVA mediated HO-1 gene regulation in human skin. Firstly, we demonstrate that Nrf2 nuclear protein accumulates in response to UVA irradiation in primary human fibroblasts, an observation possibly attributable to Nrf2 protein stabilisation mediated by UVA released heme. Secondly, in preliminary experiments, we have shown that UVA radiation enhances expression of the Bach1 gene several-fold whereas heme exposure results in no change in expression. Up-regulation of Bach1 by UVA may involve a feed-back mechanism and result from UVA mediated photochemical destruction of existing Bach1 protein bound to heme. This phenomenon may also be related to the development of refractoriness to re-induction of the HO-1 gene by

UVA observed previously (this laboratory). Strong down-regulation may develop in the presence of newly synthesised Bach-1 in a situation where free heme is destroyed by newly synthesised HO-1. We propose that altered levels of free heme that accumulate after UVA irradiation will play a crucial role in the dynamic exchange in the occupation of MARE sites by Bach and Nrf proteins bound to Maf and strongly influence UV-mediated HO-1 gene expression.

Acknowledgements: the authors acknowledge collaboration with J. Alam (Ochsner Clinical Foundation, New Orleans, USA) in preliminary experiments with Nrf2 and the Association for International Cancer Research (UK) for funding.

PI30

UVB induced sunburn cell formation of human keratinocytes is mediated through the Ask-1-p38MAPK cascade

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The UVB fraction of solar radiation is a complete carcinogen and apoptosis of UVB-damaged keratinocytes is a fail-safe mechanism, which eliminates potentially mutagenic keratinocytes. Insight into the molecular mediators triggering UVB-induced sunburn cell formation, and the disturbed pathways underlying the sunburn resistant and carcinogenic phenotype, is a requisite to find effective anticancer therapeutics. We have shown that p38MAPK is a strong pro-apoptotic mediator of the keratinocyte response to UVB as it engages the mitochondrial death pathway, by triggering mitochondrial Bax translocation and subsequent cytochrome c release in the cytosol. The mechanism by which UVB enrolls the sequential activation cascade leading to p38MAPK activation remained unclear. In the present study we investigated the role of Apoptosis Signal Regulating Kinase-1 (Ask-1) as putative MAPKKK in the cascade leading to the activation of p38MAPK in UVB-treated keratinocytes. Ask-1 is a redox protein that becomes activated when reactive oxygen species (ROS) dissociate it from its negative regulator thioredoxin-1 and UVB is a potent inducer of ROS. HaCaT cells stably transfected with wt-Ask-1 displayed enhanced mitochondrial apoptosis after UVB irradiation as indicated by the more pronounced translocation of Bax to mitochondria and secondarily release of cytochrome c observed in the wt-Ask-1 cells compared to mock-transfected cells. Consistent with a role of Ask-1 as upstream regulator of the pro-apoptotic p38MAPK cascade, the extent of p38MAPK activation following UVB in wt-Ask-1 overexpressing cells was also found to be remarkably enhanced. Hence, these observations suggest that in human keratinocytes Ask-1 senses the oxidative damage inflicted by UVB and converts it into a signal that starts up the intrinsic apoptotic machinery through the p38MAPK mediated activation of Bax. The role of the Ask-1-p38MAPK cascade in skin carcinogenesis will be discussed.

PI31

Efficacy of different UV emitting sources in the induction of T cell apoptosis

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Objective: one of the major mechanisms of UVB immunosuppression in the treatment of different skin diseases is thought to be an apoptosis-inducing effect on T cells infiltrating the skin. We examined the T cell apoptosis-induction capacities of different UV light sources.

Methods: 4 different polychromatic UV light sources with and without filters were used to irradiate peripheral blood mononuclear cells. The extent of apoptosis was measured by Apo2.7 antibody staining and flow cytometry.

Results: the xenon chloride (XeCl) laser proved to be the strongest apoptosis-inducer. The use of a phthalic acid filter eliminated UV radiation almost completely below 300 nm, which resulted in a severe decrease in the apoptosis-inducing capacity of different UVB sources. Using the results of the measurements with polychromatic UV light sources, the wavelength dependence of UVB light for the induction of T cell apoptosis was also determined. The regression line of the action spectrum demonstrated a continuous decrease from 290 nm to 311 nm.

Conclusion: the decreasing trend of this action spectrum is similar to that for erythema induction and thymine dimer formation. The explanation for the similarities might be that all of these processes are mostly mediated by UV-induced DNA damage.

PI32

Induction of CCL21/SLC and dendritic cell activation by photodynamic therapy

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Tumor response to photodynamic therapy (PDT) involves a complex interplay between direct cytotoxicity to tumor cells and secondary damage as a result of the effects of PDT on the vasculature and stimulation of the host inflammatory response. Pre-clinical and clinical studies have suggested that the combination of direct and indirect effects of PDT culminate in an activation of host anti-tumor immune responses. Induction of an immune response is dependent upon the maturation and activation of antigen presenting cells, i.e. dendritic cells. We show here that *in situ* PDT treatment of tumors results in increased expression of CCL21/SLC (secondary lymphoid chemokine) in tumor draining lymph nodes, which important in dendritic cell migration to secondary lymphoid organs. The increase in CCL21/SLC correlates with an increase in activated dendritic cells as measured by flow cytometry and intracellular staining for IL-12. We also show that dendritic cells present in the tumor draining lymph nodes of PDT treated mice exhibit increased functional activity as measured by an enhancement in ability to stimulate T cell proliferation and activation. These studies suggest that one mechanism by which PDT is able to activate the host anti-tumor immune response is through activation of dendritic cells.

PI33

Induction of tolerance by UV-induced, Platelet Activating Factor-stimulated, IL-10 secreting, B cells

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The immunosuppressive properties of solar UV radiation is a major risk factor for human skin cancer induction. UV exposure also suppresses the immune response to a variety of microbial antigens, indicating that UV-induced immune suppression has the potential to adversely affect human health and well being. Draining lymph nodes cells isolated from UV-irradiated and hapten-sensitized mice induce systemic immune suppression and tolerance when injected into normal recipient animals. Previously, we showed that platelet-activating factor (PAF), a lipid mediator of inflammation released by UV-irradiated keratinocytes, induces systemic immune suppression. Here we asked whether PAF is also responsible for UV-induced immune tolerance. As expected, transferring FITC⁺ lymph node cells from UV-irradiated mice into normal recipient animals induced tolerance. What was unexpected was the finding that the FITC⁺ cell that induced tolerance in the recipient mice was an IL-10-secreting, B220⁺ B cell. When FITC⁺ cells were isolated from the draining lymph nodes of UV-irradiated mice treated with a

selective PAF-receptor antagonist, tolerance induction was lost. These data identify a novel function for PAF in modulating immune function, activation of regulatory B cells that induce immune tolerance.

PI34

The effects of TL01 phototherapy on UVB-induced Langerhans' cell, CD11b⁺ and iC3b⁺ cell trafficking in polymorphic light eruption (PLE)

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Recent *in vivo* studies suggest that reduced UVB-induced immunosuppression may be involved in the aetiology of PLE. Photoimmunosuppression is characterised by Langerhans' cell (LC) loss and a concurrent influx of CD11b⁺ macrophage-like cells. If stimulated with the CD11b ligand, iC3b, these cells produce IL-10 and suppress Th-1 responses. We have investigated whether CD1a⁺LC, CD11b⁺ and iC3b⁺ cell trafficking is aberrant in PLE and whether TL-01 phototherapy modulates these cell responses.

10 PLE patients and 10 healthy volunteers were irradiated on buttock skin with a series (21-200mJ/cm²) of broad-band (TL-12) UVB. 24h later, MEDs were recorded and punch biopsies taken from the 200mJ/cm² site and a non-irradiated site. A subset of 7 PLE patients underwent TL-01 phototherapy (5wk; 3x/wk; starting at 70% TL-01 MED; 20% increments) after which the MED testing/biopsy protocol was repeated. CD1a⁺LC were counted in epidermal sheets. CD11b⁺ (% area) and iC3b⁺ cells were scored in cryosections.

Before phototherapy, both healthy (n=10) and PLE (n=10) subjects showed CD1a⁺LC loss 24h after 200mJ/cm² TL-12 (p<0.001) with depletion being inversely correlated with TL-12 MED (R²>0.58). In healthy and PLE subjects CD11b⁺ staining and iC3b⁺ cell number increased 24h after 200mJ/cm² TL-12 (p<0.05). In PLE subjects (n=7), TL-01 phototherapy *per se* reduced CD1a⁺LC (p<0.04) but had no significant effect on CD11b⁺ expression or iC3b⁺ cell number. 200mJ/cm² TL-12 augmented CD1a⁺LC loss (p<0.02) and increased iC3b⁺ cells and CD11b⁺ staining (p<0.05).

These results confirm that prior to phototherapy, LC responses to UVB challenge are normal in PLE. In all subjects LC loss was dependent on individual MED. CD11b⁺ and iC3b⁺ responses to UVB were not significantly abnormal in PLE. TL-01 phototherapy reduced LC but had no effect on CD11b⁺ and iC3b⁺. Interestingly, UVB challenge after phototherapy further depleted LC and upregulated CD11b⁺ staining and iC3b⁺ cells suggesting that photoimmunosuppressive mechanisms could still be stimulated.

This study was part of the EU funded 'SUNALL' project, QLK4-CT01-00115. www.sunall.org

PI35

Directing the immune system by light

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The aim of our work has been to direct immunity remotely by light, in a highly efficient and specific manner, such that immunity may be stimulated in the body when and where required. Our approach is based on the technology we have previously developed enabling the light activation of antibodies¹. We have chosen the T-cell as the target for this work. Anti-T-cell antibodies are rendered photo-reversibly inactive such that their activation, and thus subsequent T-cell activation, becomes light-dependent. Employing established markers of T-cell activation we have demonstrated that T-cells may be activated remotely by light in the presence of these light-dependent antibodies. Furthermore, we have successfully utilised this ability to investigate tumour cell killing in *in vivo* preclinical studies. A direct and potentially far-reaching application of this

work is its application to the clinical use of bispecific antibodies to retarget T-cells to tumours. The use of such bispecific antibody constructs, incorporating both T-cell and anti-tumour binding activities, has faced two critical challenges. First, there are very few (if any) truly tumour-specific antibodies and secondly, constructs containing active anti-T-cell activities are sequestered by peripheral T-cells in the body before reaching the tumours. Rendering the anti-T-cell activity inactive until illuminated impacts on both of these. The specificity of the constructs may be dramatically increased by virtue of their now being regionally specific. Premature peripheral T-cell binding and sequestration before the T-cell binding activity is required is also negated. Design of clinical studies is now being undertaken and we expect that this technology will add to the growing number of applications of photo-medicine.

References: ¹Self CH, Thompson S, Light activatable antibodies: Models for remotely activatable proteins. *Nature Medicine* (1996), 2(7), 817-820.

PI36

Modulation of contact hypersensitivity in mice by photolysis products of psoralens

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Psoralens (PS) plus UVA radiation (PUVA-therapy and photopheresis) are widely used for the treatment of a variety of skin and autoimmune diseases associated with hyperactivity of T-cell immunity. Some of the beneficial effect of PUVA-therapy and photopheresis may be due to their immunosuppressive properties. Photolysis of psoralens occurs during PUVA or photopheresis treatment. The role of PS photoproducts in immunosuppression is still not clear. We assessed effect of photooxidation products of psoralen (Ps), 8-methoxypsoralen (8-MOP) and 5-methoxypsoralen (5-MOP) on contact hypersensitivity (CHS) in mice, the experimental model of human contact dermatitis. It was found that orally administered photooxidation products of Ps, 8-MOP and 5-MOP induced suppression of the CHS to haptens 2,4-dinitrofluorobenzene and oxozalon. Immunosuppressive efficiency of photooxidation products decreased in a row: 5-MOP>8-MOP>Ps. Immune effects of photoproducts were compared with those of PUVA-treatment with 8-MOP (orally applied 8-MOP plus UVA-irradiation of skin). We found that PUVA-treatment with 8-MOP inhibits not only the afferent but as well the effector phases of the CHS. Photooxidized Ps and 5-MOP induced fluence-dependent suppression of CHS. In contrast to this photooxidized 8-MOP produced modulative effects on the CHS. Namely, application of 8-MOP preirradiated in low fluences produced nearly 100% activation of the CHS, while activation changed over to complete suppression of the CHS at higher fluences. Ps-induced suppression of the CHS was found to be adoptively transferable and antigen-specific suggesting the generation of cells with suppressive functions. The results indicate that both activation and suppression of the CHS after PUVA-treatment might be the result of production of PS photooxidation products.

PI37

Lack of antigen-specific immunity in the very early stage of photocarcinogenesis in an animal model

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Chronic irradiation of human or murine epidermis with ultraviolet B (UVB) induces clones of p53-mutant keratinocytes. Clones precede and parallel the induction of carcinomas, suggesting that they are an early stage of UVB carcinogenesis. In the absence of

UVB, these clones rapidly regress. For UVB-induced murine skin tumors and papillomas, regression is known to involve immunity. To determine whether antigen-specific immunity influences the creation, expansion, or regression of *p53*-mutant clones, we studied *Rag1* knockout mice deficient in the recombination activating gene 1 required for development of B, $\alpha\beta$ T, $\gamma\delta$ T, and NKT cells. Since tissue homeostasis could affect proliferation or persistence of clones, we also examined the effect of *Rag1* on UVB-induced hyperplasia and apoptosis. Mice were irradiated with UVB daily for 7-11 wks to create *p53*-mutant clones, and then retained in the absence of UV. After UV ended, epidermal thickness decreased and *p53*-mutant clones observed in epidermal sheets regressed, with no significant differences between *Rag1*^{-/-} and wild-type. During the initial chronic UVB irradiation, increasing irradiation time increased both the number and size of *p53*-mutant clones, with no significant difference between genotypes. We conclude that antigen-specific immunity is not involved in the initiation, expansion, or acute regression of *p53*-mutant clones.

PI38

Effect of UV irradiation on cellular responses and DNA damage of human keratinocytes harboring HPV16

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In Epidermodysplasia verruciformis and some immunocompromised patients cooperative effects of human papillomavirus (HPV) and ultraviolet (UV) radiation have been postulated in the development of non-melanoma skin cancers. The protein p53 is a critical mediator of the cellular response to genotoxic agent by inducing either growth arrest, DNA repair or apoptosis. Transforming activity of high risk HPV is associated with loss of both cell cycle control and DNA repair systems. The aim of our study was to determine the effects of UV irradiation on cellular responses and DNA damage in HPV16 infected human keratinocytes. We used two cell lines, SKv-e and SKv-l, different in their viral genome integration, *in vitro* proliferative potential, and *in vivo* tumorigenicity in mice. Firstly, we showed that the weakly proliferative and tumorigenic SKv-e cell line was more sensitive to UV-induced apoptosis, accumulated strongly p53 and p21 proteins in their nucleus and that the p21 protein nuclear accumulation resulted from a p53-dependent transcriptional activation compared to the highly proliferative and tumorigenic SKv-l cell line. Moreover, we showed for the two cell lines that UV irradiation induced the same level of CPD and (6-4) photoproducts whereas in SKv-e cell line higher level of 8-oxodG was formed. Interestingly, this higher oxidative DNA damage formation in SKv-e cell line was related with low GSH-Px activities and GSH contents. Furthermore, CPD and (6-4) photoproducts are repaired to the same rate for the two cell lines although the repair of (6-4) photoproducts was most efficiently achieved than for CPD. On the contrary, 8-oxodG was not repaired for the two cell lines. In conclusion, our results demonstrate that p53 remain inducible and functional in HPV16-infected cells and confirm a cooperative role of HPV and UV in the transformation of keratinocytes and the development of cutaneous carcinomas by allowing DNA damage accumulation in HPV infected cells.

PI39

The UV(B) fingerprint dominates the PTCH mutation spectrum of psoralen plus UVA-associated basal cell carcinomas

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Basal cell carcinoma (BCC) represents the most frequent human skin cancer. Epidemiologic studies have clearly revealed that in the general population BCCs are most often linked to chronic sun exposure. In psoralen and UVA (PUVA)-treated psoriasis patients BCCs also occur at an increased incidence but the exact etiology and pathogenesis in those subjects is unclear at present. Photochemical reactions of psoralen with DNA are well characterized and particularly the TpA sequence is the common cross-linking binding site of psoralen at the DNA level; consecutively, as shown *in vitro* and in animal studies mutations at TpA sites are considered to be of PUVA origin. Interestingly however, in a previous study of the p53 tumor suppressor gene we had found that PUVA-associated BCCs most frequently carried the UV(B) fingerprint, i.e. C-to-T or tandem CC-to-TT transitions at dipyrimidine sites. In this study, we performed a molecular mutation analysis of the patched (PTCH) tumor suppressor gene in PUVA-associated BCCs. We analyzed 8 BCCs from 4 psoriasis patients treated with long-term PUVA and high cumulative UVA doses. DNA was extracted from archived paraffin-embedded sections. Polymerase chain reaction (PCR) was used to amplify DNA of exon 2 to 23 of the PTCH gene and the adjacent intron region. To detect mutations direct automated multicolor fluorescent labeling capillary electrophoresis sequencing was performed. Four of 8 BCCs showed a total of 9 missense and 1 nonsense exon mutations as well as 3 additional intron mutations. The majority of mis/nonsense mutations (8/10; 80%) was of UV(B) fingerprint (C-to-T transitions at dipyrimidine sites). There were no mutations at TpA sites. We conclude that environmental and/or therapeutic exposure to UV(B) radiation seems to be the main factor in PUVA-associated BCC mutagenesis whereas PUVA treatment itself does not play a major direct role.

PI40

Activating c-Kit exon 11 mutation is absent in Merkel cell carcinoma

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Merkel cell carcinoma (MCC) is a rare but very aggressive skin tumor of putative neuroendocrine origin, primarily affecting elderly persons at sun-exposed body sites. The exact causes and molecular mechanisms of MCC formation are not known at present. A candidate gene is the proto-oncogene c-Kit, which encodes for a transmembrane receptor tyrosin kinase (ckit/CD117) related to the platelet-derived growth factor (PDGF)/colony stimulating factor 1 (CSF-1) receptor subfamily. The fact that strong c-Kit expression has been reported in primary and metastatic MCC makes the c-Kit gene a suspect candidate in this type of tumor. C-Kit is involved in activation of cell proliferation and is thought to play an important role in melanogenesis, hematopoiesis, and carcinogenesis. In other cancers, most c-Kit mutations were found in the hot-spot region of exon 11, involving codons 550 - 560. Previous studies give evidence that a point mutation at codon 560 is capable of inducing constitutive activation of c-Kit product, and raise the possibility that this activating mutation may be involved in oncogenesis of some cell types, including hematopoietic stem cells and mast cells. In this study, we analyzed MCC from 14 patients (12 females and 2 males; age range, 70 to 88 years). Direct sequence analysis of c-Kit exon11 revealed no mutations. Although the results of the present study do not exclude the possibility of c-Kit mutations in exons other than 11, the data do not support the hypothesis that activating mutations in c-Kit exon 11 play a role in the pathogenesis of MCC.

PI41**UVA sensitivity in Smith-Lemli-Opitz syndrome: possible involvement of cholesta-5,7,9(11)-trien-3 β -ol**

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Smith-Lemli-Opitz syndrome (SLOS) is a severe developmental disorder caused by a deficiency of 7-dehydrocholesterol reductase. SLOS homozygotes exhibit a marked deficiency of cholesterol in plasma and tissues with a concomitant increase in 7-dehydrocholesterol (7-DHC). In 1998 UVA photosensitivity was recognized as part of SLOS (*Br. J. Dermatol.* 138, 885); maximal erythema response was seen at 350nm. Because 7-DHC has no UVA absorption it seems unlikely that it can be the cause of SLOS photosensitivity. We have previously shown that UVA phototoxicity in earthworms is due in part to the presence of cholesta-5,7,9(11)-trien-3 β -ol (9-DDHC) (*Photochem. Photobiol.* 66, 316, 1997). Furthermore, 9-DDHC has been detected in plasma from SLOS patients (*J. Lipid Res.* 37, 2280, 1996). Because 9-DDHC has strong absorption in the UVA range ($\epsilon \sim 15,000$ @ 324nm) we have studied its photobiology to determine whether it may be involved in SLOS photosensitivity. Human HaCaT keratinocytes were treated with 7-DHC or 9-DDHC (0-10 μ M) in PBS/glucose (10 mM) and then immediately exposed to 15 J/cm² UVA. Following exposure cells were serum starved for 12 hours and their viability measured using the MTS assay. Exposure to UVA and 2, 4 or 10 μ M 9-DDHC resulted in 5%, 44% and 88% decreases respectively in viability (compared to dark controls). No damage was observed in 7-DHC/UVA or UVA exposed cells. UVA/9-DDHC treated cells exhibited an increase in reactive oxygen species detected by CMH₂DCFDA. Upon UVA irradiation 9-DDHC generated singlet oxygen (quantum yield ~ 0.01 in MTBE) and superoxide in MeOH. These findings suggest that reactive oxygen species generated by 9-DDHC may play a role in the UVA skin photosensitivity of SLOS patients.

PI42**Quantitative and temporal differences in UVR dose-dependent skin responses in hairless rodents (mice, rats, and guinea pigs)**

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The UVR dose required to produce a threshold skin response (Minimal Erythema Dose; *observational MED*) was determined in animals of four genetic origins (albino hairless mice, pigmented hairless mice, female albino hairless rats, and albino hairless guinea pigs). Each animal (10 mice or rats per sex and 5 guinea pigs per sex) was exposed to a series of six UVR doses previously bracketed as appropriate to its respective genetic origin. All animals were immobilized with light anesthesia. An aluminum foil mask with six holes, each with a diameter of approximately 4 mm serving to define the irradiation sites, was placed over each animal before UVR exposure. A single UVR exposure was delivered to each irradiation site. The site defined by the mask was marked using an indelible marker to assist in locating the exposure sites. The irradiation source was a Solar Light Compact Arc high intensity solar simulator (Solar Light Company, Philadelphia, PA) with a WG 320 Schott glass filter (1 mm) coupled to an Oriel light pipe (emission restricted to UVB and UVA). The radiant intensity of the source was monitored with a Solar Light PMA 2100 radiometer. All exposed spots were examined immediately and at 1, 4, 24, 48, and 72 hours post exposure, and for each animal, the lowest dose of UVR to produce a visible response was tabulated. Means and standard deviations were calculated for each animal at each observation period. Among the three species, skin responses differed quantitatively and temporally. One available comparison metric is the number of Standard Erythema Dose units (SED; *CIE Standard 007/E-1998*) required to induce a threshold response. Irradiances associated with an observational MED were highest in

the hairless rats (about 20 SED), next in the hairless guinea pigs (about 15 SED) and lowest in the pigmented and albino hairless mice (about 3 SED). Threshold responses appeared earliest in hairless guinea pigs and latest in hairless rats.

PI43**Winter eye protection for ultraviolet radiation**

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The need for protection of the eye from excessive solar ultraviolet radiation (UVR) is determined largely by ground reflection. The eyes normally look downward and the upper lid and brow-ridge shield the cornea from direct overhead sunlight exposure. The typical global (diffuse + direct) biologically weighted solar ultraviolet radiation measured by solar monitoring stations during the summer months is of the order of five to ten times the exposure measured in winter months. The accumulated UV radiant exposure or peak UV irradiance during a typical fall or spring day may be of the order of 1/3 the summer irradiance as measured on a flat horizontal surface. Studies of ocular exposure show that the cornea is seldom exposed to the direct rays of the sun except near sunrise and sunset. The reflected radiant energy varies enormously with ground surface. The typical reflectance of green grass is only of the order of 1%, whereas, construction surfaces, road surfaces and sidewalks, typically reflect about 10% UV-B; sea surf reflects 20% or more, and the reflectance of fresh snow is approximately 85%. Photokeratitis ("snow-blindness") rarely occurs except when snow is on the ground—and yet this acute corneal injury occurs when the sun is relatively lower in the sky with less ambient solar UVR than in the summer (when photokeratitis is rare). These facts are traditionally used to illustrate that the dominant factor in ocular exposure to UVR is the ground surface. In mid-winter at most snowy latitudes, the ambient UVR global irradiance is not sufficient to produce corneal injury, but near the spring equinox the solar zenith angle rapidly decreases (the solar elevation angle rapidly increases) and conditions are favorable for photokeratitis. Traditional age-old eye protection included veils and slit goggles to protect against both the wind and sunlight. Today's ski-goggles emphasize protection by spectral filtration. Sunglasses filters provide strong UVR filtration, but without a goggle frame, the wearer may readily experience photokeratitis.

PI44**UV-A irradiance at typical indoor life space**

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Since the late 1990s there has been concern about increasing UV-B radiation at the earth's surface, which leads to serious problems for human life and ecosystems. Solar UV-A (320 – 400 nm) radiation is independent of ozone depletion, and continuously reaches the surface of the Earth from the sun. Moreover, UV-A radiation is emitted from artificial light sources. Recently, interest has been intensified regarding UV-A harmful effects on human health. On the other hand, the useful effects of UV-A are also well known, such as color forming of fruits and vegetables; bonding agent activation in the building industry; and photocatalytic reaction, antibacterial, antipollution, degradation of chemical substances. The need for measurement of UV-A irradiance has been increased steadily. To study and evaluate UV-A irradiance under indoor lighting environment, the optical radiometry of UV-A irradiance indoor was conducted at typical Japanese houses.

UV-A irradiance at living, dining, kitchen, study, rest room and bathroom was measured with a new UV-A radiometer (C9641: Hamamatsu Photonics), which is developed for efficacy evaluation of photocatalytic properties. To estimate effect of outdoor solar UV-A irradiance on UV-A irradiance indoor, global solar UV-A

irradiance was measured by commercially available UV-A radiometers (MS-210A and MS-211I; EKO).

At daytime (without room lighting), UV-A irradiance indoor depended on global solar UV-A irradiance pass through window, and the largest UV-A irradiance indoor was recorded at sunny place near by window. On the other hand, UV-A irradiance indoor at not sunny place was 1/100 or less of global solar UV-A irradiance and depended on direction of window. At night, UV-A irradiance of interior lighting was 1/1000 or less of maximum global solar UV-A irradiance, and depended on type and place of luminaire, and light source using in room.

PI45

Efficacy of RGD-porphyrins on murine tumoral melanocytes: preliminary results

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Background: Photodynamic therapy (PDT) is mediated by reactive oxygen species after activation of a photosensitizer by light. PDT remains controversial for melanoma treatment. This is due the poor light penetration of the activating 630nm light into melanin-rich tissue with sodic porphyrin PDT. To increase their specificity RGD function has been grafted on porphyrins. RGD binds specifically on cell surface integrin $\alpha 5\beta 3$ which is expressed by tumour melanocytes and endothelial cells. We report herein some preliminary results concerning the efficacy of newly synthesized porphyrins on murine melanocytes (B16F10) and bovine endothelial cells (EJG).

Material and Methods: Para-Triglycosyl-RGD porphyrin and Ortho-TG-RGD synthesis have been previously published. B16F10 and EJG cells have been cultured in wells. Porphyrins were added after 3 days. Irradiation was performed with 10J/cm², blue light, (450 nm Wavelength) after 3 hours incubation. Colorimetric MTT test was performed after irradiation, at day 1, 2, 3, and 4 to determine cytotoxic and phototoxic porphyrin concentrations.

Result: oTG-RGD porphyrin is phototoxic and not cytotoxic at 10⁻⁵M concentration on B16F10, inducing 60% of cell death at day 2. pTG-RGD porphyrin is phototoxic and not cytotoxic on B16F10 at 10⁻⁴M concentration, inducing 90% cell death. At these concentrations porphyrins are cytotoxic on EJG. pTG-RGD porphyrin is phototoxic and not cytotoxic on EJG (10⁻⁹M). Percentage of cell mortality is comparable to rate of cell mortality with Photofrin (15µg/ml).

Conclusion: these results suggest that PDT using new RGD-porphyrins is active on tumour melanocytes. Various types of porphyrins are being synthesised and evaluated to further precise their activity and specificity.

PI46

Photodegradation of folic acid during extracorporeal photochemotherapy

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Photodegradation of folic acid by UV radiation is a well documented photochemical reaction and decreased serum levels of folic acid have been found in patients receiving photochemotherapy (PUVA). During extracorporeal photochemotherapy (ECP) leukocytes and plasma are treated with 8-MOP/UVA. In the current study we investigated whether ECP leads to the photodegradation of folic acid in the extracorporeal system.

Thirty patients undergoing ECP on two consecutive days were enrolled into the study. Heparinized plasma was obtained from the extracorporeal system before and immediately after UVA exposure

on both treatment days. Healthy donor plasma was exposed to 8-MOP and increasing doses of UVA *in vitro*. Folic acid (5-methyltetrahydrofolate) was determined by a radioassay (Simultrac-SNB, Becton-Dickinson). Vitamin B12 and homocysteine, not undergoing photodegradation, were used as control parameters.

On the first day of ECP folic acid levels decreased from 7.11 ± 5.8 nmol/l to 3.9 ± 2.6 nmol/l (mean ± SD, p<0,005) after UVA exposure. On the second day the reduction was from 5.9 ± 4.9 nmol/l to 3.4 ± 3.0 nmol/l (mean ± SD, p<0,005). This correlates to a decrease after UVA of 45.1 % and 42.4 %, respectively. This effect could be reproduced *in vitro* demonstrating that folic acid reduction depends on UVA dose but not on the presence of 8-MOP. Only minor changes were observed for vitamin B12 and homocysteine.

From these results we conclude that extracorporeal exposure of plasma to UVA during ECP leads to photodegradation of folic acid. Further investigations are required to determine the biological effects of folate photoproducts and whether clinically relevant loss of folic acid might be a consequence of ECP.

PI47

Efficacy of Variable Pulsed Light in the treatment of hypertrichosis in 103 patients

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Variable pulsed light (VPL, Energist Ltd., UK) is an alternative to Intense Pulsed Light (IPL) in the treatment of hypertrichosis. To document its efficacy we evaluated the results in 103 consecutive otherwise healthy previously treated and untreated volunteers (Skin Types II-VI): 97 women and 6 men. Treatment was performed with a variable pulsed 610nm light source with an off delay time of 1ms to 20ms; spot size was 5cm x 1cm. Clinical evaluation was performed using the Teach Screen Monitoring System (Foto-Finder, Mediscope, Germany). Prior to every treatment procedure the area was covered with a refrigerator cooled coupling gel (4Deg. C.; Energist Ltd., UK). Determination of fluence used was determined on the individuals' tolerance to pain; this to a great extent depended on the specific skin type and coarseness. The average fluence used was 37J/cm² (range 13-48 J/cm²). Eighty six percent of the areas treated showed hair clearance of over 50%, thirty four percent showed hair clearance of over 75% while fifty two percent had clearance between 50 and 75%. The observed side effects included erythema (47%) and leucotrichia (11%); these side effects were minimal and in no instance significant enough to cause interruption of treatment. Pigmentary changes were not observed. The variable pulsed light system used in this study presents itself as a safe and effective method for hair removal due to hypertrichosis for medical and/or cosmetic indications.

PI48

Impact of polymorphic light eruption on quality of life across four seasons and at a range of latitudes in Europe

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Polymorphic Light Eruption (PLE) is one of the commonest skin disorders affecting Europeans, yet effects of photosensitivity on life quality have not previously been reported. We wished to examine

the impact on QOL in sufferers living at a range of latitudes in Europe.

A prospective study was performed in 237 PLE patients attending hospital dermatology departments in Athens, Besancon, Leiden, Manchester and Turku (latitudes 37.976 – 60.455). Patients completed a validated questionnaire, the Dermatology Life Quality Index, and a global visual analogue score (VAS), relating to their experience in the previous week. This was performed 4 times, for the first week of April, July, October and January in 2003-2004. The response rate was 69-81% over the 4 seasons. The mean age of the population was 43 years (range 18-74); female to male sex ratio 4:1. The DLQI showed a strong seasonal pattern, the mean index for all countries being: April $3.33 \pm SE 0.3$, July 5.94 ± 0.4 , October 2.59 ± 0.3 and January 1.36 ± 0.2 . Significant differences were observed for January vs April, January vs July, April vs July, and July vs October ($p < 0.01$). DLQI correlated highly with VAS in all 4 seasons ($r = 0.73-0.85$, $p < 0.001$), but did not show any correlation with age, sex or skin type. Interestingly, no correlation was seen between DLQI and location, patients suffering equally at the extremes of latitude studied.

Hence PLE causes significant seasonal disability in sufferers across Europe; more efforts are needed to better understand and manage this ultraviolet-induced disorder.

This project was funded by the European Union Framework V Programme, project no. QLK4-CT01-0015.

PI49

The effects of TL-01 phototherapy on erythema and provocation responses in polymorphic light eruption (PLE)

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Narrow-band UVB (TL-01) phototherapy is reported to be an effective prophylactic treatment for PLE although its ability to cause photoadaptation of the skin against UVB-induced erythema and UVA-provoked PLE has not been objectively quantified.

10 PLE patients and 10 healthy volunteers of skin types I – III were irradiated on photoprotected buttock skin with geometric series of 10 doses of broad-band TL-12 and TL-01 radiation. Dia-stron® erythema meter readings were taken at 24h on irradiated and control sites. Reflectance data was analysed to yield (i) $D_{0.025}$ as an objective measure of minimal erythema dose (MED) and (ii) the maximal slope of the erythema curve (SEC). The upper forearms of the same PLE patients were exposed to 20 J/cm² broadband UVA, for a maximum of 3 successive days, and PLE responses recorded 24h after each irradiation. 7 PLE patients then underwent a five week course of TL-01 phototherapy (3x/wk; starting at 70% TL-01 MED; 20% increments) after which the above investigations were repeated.

Before phototherapy, there was no difference in TL-12 induced $D_{0.025}$ ($p > 0.85$) or SEC ($p > 0.14$) between normal and PLE subjects. However, both TL-01 induced $D_{0.025}$ ($p = 0.04$) and SEC ($p = 0.01$) were significantly lower in PLE patients. Following phototherapy, $D_{0.025}$ values increased significantly by a factor of 2.5 for TL-12 ($p = 0.01$) and 1.9 for TL-01 ($p = 0.01$) whilst SEC for both sources remained unchanged ($p > 0.5$). Significant photoadaptation to UVA-induced PLE was shown in 6/7 patients ($p < 0.01$) with a mean increase in cumulative UVA provocation dose of $> 30 \text{ J/cm}^2$.

These data suggest that PLE subjects may have abnormal erythema responses to 311nm (TL-01) radiation. We have also shown objectively that TL-01 phototherapy significantly photoadapted skin against UVA-provoked PLE and offered a twofold protection against UVB erythema. *This study was part of the EU funded 'SUNALL' project, QLK4-CT01-00115. www.sunall.org*

PI50

Acute UV irradiation and heat shock induced exon 26A mRNA and protein expression of elastin in human skin *in vivo*

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Photoaged skin contains elastotic materials in the upper reticular dermis. This phenomenon is commonly known as solar elastosis. In our previous report, we demonstrated that UV irradiation induced tropoelastin mRNA expression in the epidermis of human skin *in vivo* and also in the cultured keratinocytes *in vitro*. It is known that the primary transcript of elastin undergoes extensive alternative splicing resulting in the translation of multiple heterogeneous protein isoforms. In this study, we found that UV irradiation and heat treatment increased the levels of elastin transcript containing exon 26A and its encoding elastin isoform (26A protein) in the epidermis of human skin *in vivo* and in cultured human keratinocytes *in vitro* by nested RT-PCR, Western blot analysis. It was also shown that elastin transcript containing exon 26A was increased in the forearm (sun-exposed) skin of elderly persons, compared with buttock arm (sun-protected) skin of the same individuals. These data suggest that elastin containing exon 26A peptide induced by UV irradiation and heat treatment in human skin *in vivo* plays some roles in the development of solar elastosis.

PI51

Photochemical properties of aged human RPE melanosomes

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Human RPE melanosomes exhibit age-dependent aerobic photoreactivity. To date, the molecular nature of the chromophores responsible for the observed photoreactivity remains unknown.

The main goal of our study was to identify key constituents of RPE melanosomes responsible for their photoreactivity by analyzing photochemical properties of chloroform-soluble (CSM) and chloroform-insoluble (CNSM) fraction of the melanosomes.

RPE melanosomes from donors: 10 - 20, 45 - 55 and 80 - 89 year old, and from bovine eyes, were purified by ultracentrifugation in a discontinuous sucrose gradient. Electron spin resonance (ESR) oximetry and ESR spin trapping were used to determine oxygen uptake and generation of superoxide anion in suspension of melanosomes irradiated with blue light. Photoreactivity of human and bovine CSM was analyzed in solution using organic solvent and in liposomes by time-resolved singlet oxygen phosphorescence at 1270nm, ESR oximetry and by HPLC-EC(Hg).

Our data show that intact and triton X-100 treated melanosomes have comparable photoreactivity, while photoreactivity of melanosomes treated with chloroform/methanol is significantly reduced. Human CSM and CNSM, unlike bovine melanosome fractions, were photoreactive and such a photoreactivity increased with age. When irradiated with blue light, human CSM generated singlet oxygen.

We have demonstrated that the increased photochemical reactivity of aged RPE melanosomes result from an accumulation of chloroform-soluble photosensitizing compounds and chemical modifications of the melanin and/or proteinaceous constituents of the pigment granules.

PI52**Modulation of oxidative damage, MAPK activation and cell death in neuronal cells by the fermented papaya preparation**

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Oxidative and nitrosative stress mechanisms are involved in several biological and pathological processes including aging, cancer, Alzheimer's disease and Parkinson's disease (PD), prompting the suggestion that neurodegenerative processes can potentially be modulated by treatment with free-radical scavengers and antioxidant. The delineation of biochemical pathways involved in neuronal cell death due to oxidative stress may aid in the development of drugs and/or nutraceuticals for the treatment of various neurodegenerative diseases. Emerging data point to the involvement of the MAPKs pathways on the modulation and regulation of the exogenous induction of oxidative stress. Thus knowledge of the molecular mechanism by which specific antioxidant compound act at the level of intracellular gene cascade will facilitate this endeavour. The ability of fermented papaya preparation (FPP) to prevent H₂O₂-dependent cell death in PC12 was tested by the MTT assay and Comet assay. H₂O₂ insult was challenged with FPP. The pre-treatment for 23h with FPP followed by 1h of H₂O₂ incubation at the concentration of 250µM, resulted in increased cell viability. This correlated with a decrease of DNA damage. Protein analysis using Western blot revealed that in the presence of 250 µM of H₂O₂, FPP acted as a p38-MAPK and Akt specific inhibitor. Thus FPP may play a protective role in rescuing oxidative stress induced apoptosis, likely by activating antioxidant intracellular pathway involving p38 MAPK genes cascade.

PI53**Effect of ageing on the expression of cell defence genes after UVA irradiation of human male cutaneous fibroblasts using cDNA arrays**

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Ultraviolet A (320-400 nm) generates reactive oxygen species (ROS) that are thought to be implicated in ageing. ROS cause oxidative alterations on cell constituents, and damage accumulation can lead to mutations in DNA. DNA mutations and ageing process modulate the gene expression. Nevertheless, the results are often controversial and/or incomplete. In this study, we have documented the modifications in gene expression during ageing process as well as the cell response to an oxidative stress. For this purpose, we used a cDNA macroarray containing 82 genes related to cell defence, essentially represented by antioxidant and DNA repair proteins. Ageing-associated gene expression was assessed in normal male skin human fibroblasts from three age groups: children (n=4), adults (n=4) and elderly (n=3), at the basal state and after a 5 J/cm² UVA irradiation. Analysis revealed that 22 genes were never detected, whereas certain were always expressed such as those related to antioxidant defence, extracellular matrix (ECM) regulator and XPC. Transcripts related to ECM, MMP1 and MMP3 increased with age and after UVA irradiation, independently of age. The transcripts involved in the redox status control (TXN and APEX) decreased as a function of age, at the basal state and after

irradiation, respectively. Most of transcripts involved in DNA repair were not detected but repression of POLD1 in the adult group and induction of XRCC5 and LIG4 were observed after UVA irradiation, as a function of age. In the basal state, the transcript of GAS1, regulator of cell cycle arrest in G1 phase was found to be decreased with age. HMOX1 increased after UVA irradiation. In conclusion, decrease in expression of some antioxidant system, cell cycle control gene and extracellular matrix enzymes, particularly after UVA exposure could participate to the occurrence of photoaging.

PI54**Can broad-spectrum sunscreens prevent the secretion of proinflammatory cytokines in human keratinocytes when exposed to the phototoxic drug lomefloxacin and UVA radiation?**

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Phototoxic reactions due to the interaction of certain fluoroquinolones such as lomefloxacin (LOM) and ultraviolet A (UVA) radiation have been established in the literature. Skin is naturally exposed to UV radiation from the sun or from artificial sources where UVA represents the most important component.

Inflammation is one of the responses to UV damage and is initiated by the release of proinflammatory cytokines. Studying the efficacy of broad-spectrum sunscreens to protect human keratinocytes treated with the antibiotic drug LOM and exposed to UVA could certainly be helpful to prevent phototoxic reactions in people treated with such antibiotics. In the present study, keratinocytes were treated with increasing concentrations of LOM for 1 hour and exposed to 15 J/cm² of UVA (an exposure equal to 40 minutes in the summer in Québec City) in the presence or absence of three different broad-spectrum sunscreens available on the Canadian market. Norfloxacin (NOR), a non-phototoxic fluoroquinolone was tested in the same conditions and used as a negative control. Following drug treatment and UVA exposure, cells were incubated for 24 hours in growth medium. After incubation, cell viability and cytokine secretion were assessed. No decrease in cell viability nor increase in cytokine secretion were observed after treatment with NOR alone, NOR plus UVA or LOM alone. Treatment with LOM plus UVA had an effect on cell viability and on the secretion of proinflammatory cytokines into the medium, namely, interleukin-1α (IL-1α), tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6). IL-1α levels were significant at 15 µM LOM and continued to increase as LOM concentration increased. The release of TNF-α and IL-6 followed the same pattern as IL-1α at lower concentrations of LOM but reached a peak at 15 µM and plateaued at higher concentrations.

As expected, the addition of sunscreen between the UVA source and the keratinocytes protected the cells from the phototoxic effects of LOM as both cell viability and the levels of the studied cytokines (IL-1α, TNF-α and IL-6) remained the same as in the control cells. Therefore, the application of broad-spectrum sunscreen by individuals exposed to UVA radiation may protect them from the phototoxic reaction initiated by LOM.

PI55**Season of diagnosis is a strong prognostic factor in cancer. A possible role of sun-induced vitamin D**

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A substantial fraction of the vitamin D content in humans is generated by the action of solar radiation on 7 dehydrocholesterol (7DHC) in skin. Thus, the calcidiol (25-hydroxyvitamin D₃, 25-

(OH)D₃) level in serum is 20-100% larger in the summer than in the winter. At high latitudes, like in the Nordic countries, practically no vitamin D₃ is generated in human skin during winter, due to low UVB fluence rates.

We have recently found that this is of major significance for the prognosis of several forms of cancer: prostate, -breast, -colon, -lung cancer and lymphomas. The risk of death as estimated three years after diagnosis and therapy start is 20-50% lower for summer and autumn diagnosis than for winter and spring diagnosis. Our study includes all cancer cases diagnosed in the Norwegian population between 1964 and 2000. Risk estimates were calculated as relative risk (RR), with 95% confidence intervals, using a Cox regression model.

Our results agree with cell and animal experiments reported in the literature, as well as with epidemiological data from some countries relating survival from different malignancies with latitude and vitamin D₃ synthesis in skin.

Our finding may be related to higher endogenous levels of vitamin D₃ in the autumn, with a favourable influence on the conventional therapy.

PI56

Reaction of antioxidants with a stable colored free radical as a mean to assess their activity

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Antioxidants might have a double role in the course of photosensitization processes. They can protect biological systems from injurious oxidative reactions (Bohm et al. *J. Photochem. Photobiol.* 2001, 65, 177-83) or potentiate tumor photodynamic therapy in certain conditions (Melnikova et al. *Int. Cancer*, 2001, 88, 798-803). This dual action might be understood by considering the dynamics of antioxidant reactions with emphasis on the relative importance of hydrogen abstraction versus electron transfer mechanisms.

Such reactions have been investigated in hydroalcoholic media by using diphenylpicrylhydrazyl (DPPH^o), a stable radical that displays large change in its absorption spectrum upon reduction to the hydrazine analogue DPPH-H. Trolox (TrOH), a water-soluble analogue of vitamin E, was chosen as a standard antioxidant. Time-resolved spectra and kinetics were recorded by using a stopped-flow.

The initial step was likely to involve the phenolic group of TrOH with formation of the TrO^o radical. However, as shown by time resolved spectra recorded either by using excess of DPPH^o or TrOH, no significant amount of the TrO^o radical was accumulated. This indicated that the TrO^o radical reacted quickly with DPPH^o in a second step. The reaction stoichiometry is in agreement with this two steps process. The rate of the initial step significantly increased with the water content in the hydroalcoholic mixture suggesting predominance of an electron transfer mechanism. The rate is also increased to some extent at higher pH. The reactivity of Trolox is compared to that of other antioxidants with emphasize on kinetic parameters.

PI57

Twelve-month topical study to determine the influence of bemotrizinol (Tinosorb[®] S) on photocarcinogenesis in hairless mice

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Bemotrizinol (Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine, Tinosorb[®] S), based on the novel Hydroxyphenyltriazine technology, is an oil-soluble UVR filter with strong protection in both the UVA and UVB regions. As part of the safety assessment of Bemotrizinol, a photocarcinogenesis study was performed to

determine the potential of Bemotrizinol (50 and 200 mg/g) to influence the development or growth of skin tumors in albino hairless mice exposed to simulated solar UVR. Bemotrizinol or vehicle formulations were topically administered approximately 15 minutes before UVR exposure on Monday, Wednesday and Friday and approximately 15 minutes after UVR exposure on Tuesday and Thursday for approximately 40 weeks. After the UVR and formulation exposure phase, mice were observed for 12 more weeks. Viability and clinical observations (skin reactions, skin tumor development and body weight) were assessed at least weekly. Necropsies were performed on all mice. The vehicle formulation slightly enhanced skin tumor development or growth. Bemotrizinol in this vehicle formulation reduced the UVR-induced skin tumor response in a Bemotrizinol dose-dependent manner, as compared with mice exposed to UVR alone. Tumor prevalence, unbiased median week to tumor, tumor yield, Peto Analysis and tumor potency ratios demonstrated the slight tumor enhancement of the vehicle formulation and the protective effect of the Bemotrizinol formulations, as compared with the group only exposed to UVR. The vehicle or Bemotrizinol did not adversely affect skin reactions, clinical observations, survival, body weights or necropsy observations, as compared with mice exposed to UVR alone. Responses in male and female mice were equivalent for all tumor and clinical parameters. Thus, the Bemotrizinol formulations had no negative impact on the photocarcinogenic response to UVR exposure and reduced the potential of UVR to induce skin tumors in a Bemotrizinol dose-dependent manner.

PI58

Twelve-month topical study to determine the influence of bisoctrizole (Tinosorb[®] M-Active) on photocarcinogenesis in hairless mice

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Bisoctrizole (Methylene Bis Benzotriazolyl Tetramethylbutylphenyl, Tinosorb[®] M-Active) defines a new class of photostable, broad spectrum, insoluble organic UVR filters. As part of the safety assessment of Bisoctrizole, a photocarcinogenesis study was performed to determine the potential of Bisoctrizole (50 and 200 mg/g) to influence the development or growth of skin tumors in albino hairless mice exposed to simulated solar ultraviolet radiation (UVR). Bisoctrizole and vehicle formulations were topically administered approximately 15 minutes before UVR exposure on Monday, Wednesday and Friday and approximately 15 minutes after UVR exposure on Tuesday and Thursday for approximately 40 weeks. After the UVR and formulation exposure phase, mice were observed for an additional 12 weeks. Viability, clinical observations including skin reactions, skin tumor development and body weight were assessed at least weekly. Necropsies were performed on all mice. The vehicle formulation slightly enhanced the development or growth of skin tumors. Bisoctrizole in this vehicle formulation reduced UVR-induced skin tumor development in a Bisoctrizole dose-dependent manner, as compared with mice exposed to UVR alone. Tumor prevalence, unbiased median week to tumor, tumor yield, Peto Analysis and tumor potency ratios results demonstrated the slight tumor enhancing effect of the vehicle formulation and a protective effect of the Bisoctrizole formulations, as compared with the group only exposed to UVR. There was no adverse vehicle or Bisoctrizole-dependent effect on skin reactions, clinical observations, survival, body weights or necropsy observations, as compared with mice exposed to UVR alone. Responses in male and female mice were equivalent for all tumor and clinical parameters. Thus, the Bisoctrizole formulations did not adversely affect the photocarcinogenic response to UVR exposure and reduced the potential of UVR to induce skin tumors in a Bisoctrizole dose-dependent manner.

PI59**Is it possible to use the photodynamic effect to determine an antioxidant activity?***M. Bancirova**Department of Physical Chemistry, Faculty of Science, Palacký University, Czech Republic*

The lifetime of reactive oxygen species (ROS) is extremely short, and if a physiological acceptor does not immediately neutralize them, ROS can damage biological systems. All aerobic organisms have developed more or less complex systems to neutralize them before their potentially harmful effect is activated. And also food (vegetable and fruit, as well as wine, tea and beer) has potential or definite antioxidant capacity to neutralize them.

The photodynamic effect involves the combination of light, an organic dye – photosensitizer and molecular oxygen. Upon irradiation, free radicals and singlet oxygen are produced.

A sensitive and simple chemiluminescent (CL) method for measuring antioxidant activity was developed. The determination of the Trolox equivalent antioxidant capacity (TEAC) is based on the inhibition of CL intensity by an antioxidant (CL system involves luminol, horseradish peroxidase and hydrogen peroxide).

The aim of this work was to compare the chemiluminescent method with experiments that were done on *Paramecium caudatum* (P.c.) with different types of the photosensitizer (e.g. fluorescein, eosin, methylene blue, phthalocyanines). The experiments were done in Petri dishes with P.c. solution (density about 25 individuals in 1 ml). There were three dishes to compare: A. – contains P.c. and tea, B. – contains P.c. and photosensitizer, C. – contains P.c., photosensitizer and sample of the antioxidant (e.g. Trolox, ascorbic acid, tea).

This research was supported by the grant from Ministry of Education MSM6198959216

PI60**Photoinduced DNA cleavage and binding studies of benzophenone-based sunscreen absorbers***J. Kasavel, A.S. Sewlall, B.S. Martincigh**School of Chemistry, University of KwaZulu-Natal, Howard College Campus, 4041 Durban, South Africa*

The topical application of sunscreens is widely practised to protect healthy and photosensitive skins from the sun. Benzophenone-based chemical absorbers are widely used in these preparations to absorb UVB and shortwave UVA radiation. In view of the fact that non-steroidal anti-inflammatory drugs with the benzophenone backbone, such as ketoprofen, are able to photosensitize DNA strand breaks, it was of interest to determine whether sunscreen-active agents with the same backbone are also able to potentiate this type of damage.

The UV absorbers investigated in this study included benzophenone-1, benzophenone-3, benzophenone-4 and Uvinul DS49. Buffered aqueous solutions of the benzophenones were irradiated in the presence of DNA at wavelengths greater than 300 nm with an Osram 500 W/2 high-pressure mercury lamp in conjunction with a 10 mm thick Pyrex filter. The irradiated samples were analysed for DNA cleavage by agarose gel electrophoresis and for DNA binding by fluorescence spectroscopy and viscosity measurements. The photostability of the UV absorbers was also investigated.

From the photostability experiments conducted, it is apparent that the benzophenone-based UV absorbers were stable to photodecomposition when irradiated with UV light. The agarose gel electrophoresis experiments, however, clearly showed that benzophenone-1 and Uvinul DS49 cleave ϕ X174 DNA when irradiated with UV light typically incident on the earth's surface, while benzophenone-3 and benzophenone-4 did not. Binding of benzophenone-1 and Uvinul DS49 to calf thymus DNA was also detected by viscometry and fluorescence spectroscopy. However, this was not observed for benzophenone-3 and benzophenone-4.

PI61**The effect of antioxidants on the *para*-aminobenzoic acid photosensitised formation of singlet oxygen***A.M. Salim, B.S. Martincigh**School of Chemistry, University of KwaZulu-Natal, Howard College Campus, 4041 Durban, South Africa*

Para-aminobenzoic acid (PABA) has been shown to be capable of penetrating cells and of photosensitising the formation of thymine dimer and singlet oxygen. It is therefore potentially carcinogenic and its use as an absorber in sunscreens is no longer desirable. Since the photochemistry of PABA is well-established, the effect of antioxidants on its ability to photosensitize singlet oxygen was investigated.

Vitamin E (α -tocopherol) and vitamin C (ascorbic acid) are antioxidants that are widely used in sunscreens as absorbers of ultraviolet radiation and quenchers of singlet oxygen. The aim of this investigation was to study the effect of these antioxidants, either individually or synergistically, on the PABA-photosensitized formation of singlet oxygen in the presence or absence of thymine in an aqueous medium of pH 3.

Light of wavelengths greater than 300 nm was used to photosensitize the formation of singlet oxygen. The formation of singlet oxygen was detected by the change in absorbance at 350 nm of *N,N*-dimethyl-4-nitrosoaniline in the presence of imidazole. The parameters varied during the investigation were the concentrations of PABA and thymine, the antioxidant concentrations and the photon flux.

Both vitamins E and C can decrease singlet oxygen formation whether individually or together. The pattern changes depending on the presence of thymine, the concentrations of thymine and PABA, and irradiation time.

PI62**An investigation of the photostabilisation of sunscreen absorbers by plant polyphenols***G.J. Mturi, B.S. Martincigh**School of Chemistry, University of KwaZulu-Natal, Howard College Campus, 4041 Durban, South Africa*

Commercial sunscreen products are used to protect the skin against harmful ultraviolet (UV) radiation that can induce skin cancer at high dosage. These products contain UV filters that can reflect, scatter or absorb UV light. The chemical UV filters responsible for the absorption of UV radiation can be photochemically modified and as a result reduce the efficacy of the sunscreen formulation. This study focused on the possible use of plant polyphenols as potential stabilisers of the photo-unstable sunscreen chemical absorber, 4-*tert*-butyl-4'-methoxydibenzoylmethane (avobenzone). Potential polyphenolic photostabilisers from the Cancer Bush plant were extracted by means of various polyphenolic extraction methods. These extracts were analysed by gas chromatography (GC), high performance liquid chromatography (HPLC), UV spectroscopy and gas chromatography-mass spectrometry (GC-MS). Polyphenols were also extracted from various Rooibos teas and compared with those extracted from the Cancer Bush plant.

The Cancer Bush extracts as well as the Rooibos tea extracts together with the specific polyphenols, epicatechin and rutin, were assessed for their ability to photostabilise avobenzone. The photostability of the chemical absorber in the absence and in the presence of the polyphenol extracts was investigated by UV spectroscopy.

The polyphenol extracts offered some degree of photostabilisation for the photo-unstable avobenzone in various solvents. A photostabilisation mechanism was proposed based on the photochemical pathway followed by avobenzone that results in its photo-instability, and the properties of the polyphenol extracts investigated.

PI63**Incorporation of sunscreen-active agents in the nanospaces of layered double hydroxides***M.K. Rotich, B.S. Martincigh**School of Chemistry, University of KwaZulu-Natal, Howard College Campus, 4041 Durban, South Africa*

Sunscreen agents are classified into inorganic and organic compounds. Inorganic oxides (TiO₂ and ZnO) act as physical blockers of ultraviolet (UV) radiation, while organic compounds (e.g. 2-ethylhexyl-*p*-methoxycinnamate) act as chemical absorbers. They are used as active ingredients in commercial formulations of creams, which are applied topically to prevent harmful UV radiation from reaching the skin. However, the photostability of chemical absorbers has come under much scrutiny and is now an area of active research.

Agents such as 2-ethylhexyl-*p*-methoxycinnamate and avobenzone have been reported to form photoproducts on exposure to UV radiation. When exposed to sunlight, the molecules absorb energy making them highly reactive. These excited molecules have a high possibility of reacting with neighbouring molecules or could photodegrade, which might potentiate photocarcinogenesis.

In this study, attempts were made to stabilise the sunscreen agents by incorporating them in the nanospaces of layered double hydroxides such as zinc-aluminium hydroxide. The sunscreens agents: 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid, 2-phenylbenzimidazole-5-sulfonic acid, 2, 2'-dihydroxy-4, 4'-dimethoxybenzophenone-5, 5'-disulfonic acid and para-aminobenzoic acid were used. Success in intercalation was studied by use of X-ray powder diffraction, infrared spectroscopy, ultraviolet-visible spectroscopy and high performance liquid chromatography methods.

PI64**Photostability and photochemical analysis of commercial sunscreens***T. Bunhu, B.S. Martincigh**School of Chemistry, Howard College Campus, University of KwaZulu-Natal, 4041 Durban, South Africa*

The many deleterious effects of the sun's ultraviolet (UV) radiation on the skin are well-documented. Sunscreens have been developed to try and mitigate the acute and chronic skin damage caused by this radiation. However, the photostability of these sunscreens has been under much scrutiny as there is an alarming worldwide increase in skin cancer. This is despite a heavy promotion by medical groups and governments for the use of sunscreen products. Previous investigations have shown that some sunscreen products lose more than 50% of their efficacy on exposure to UV radiation in a period of one hour.

In this study, we have investigated the photostability of 22 commercial sun protection products. A known amount of sunscreen was smeared onto quartz plates to achieve an application density of 1 mg cm⁻² which is a more realistic estimate of what is typically used. The transmission spectra of these samples were recorded before and after exposure to solar radiation in Durban, South Africa. The actinic flux incident on the samples was measured by means of chemical actinometry (valerophenone actinometer). Changes in the transmission profiles of the sun care products during the course of exposure were monitored at set time intervals. Since some sunscreen agents can cause photo-allergic reactions and photo-contact dermatitis, regulations limit the permissible amounts of these active ingredients in a formulation. Hence, quantification of the UV-filters was carried out by normal-phase high performance liquid chromatography with a cyanopropyl silica-bonded 5CN column.

PI65**Computational studies of the photodimerisation of 2-ethylhexyl-*para*-methoxycinnamate***W. Waud, H.G. Kruger, B.S. Martincigh**School of Chemistry, Howard College Campus, University of KwaZulu-Natal, Durban 4041, South Africa*

Commercial sunscreens contain active ingredients which can either absorb or physically block ultraviolet (UV) radiation. The most commonly used chemical absorber in commercial sunscreen products is 2-ethylhexyl-*p*-methoxycinnamate (EHMC), which is an absorber of UVB radiation.

Irradiation of dilute (~ 10⁻³ M) solutions of EHMC with wavelengths of light greater than 300 nm results in E, Z photoisomerisation leading to a photostationary equilibrium mixture.¹ However, concentrated or pure solutions of EHMC upon prolonged irradiation show additional photoproducts.² These have been isolated by preparative high performance liquid chromatography (HPLC) and characterised by nuclear magnetic resonance (¹H NMR) spectroscopy, which identifies them as [2 + 2] cycloadducts.

In addition, *ab initio* molecular orbital calculations have been used to investigate the structures and the transition states of the various dimers resulting from the cycloaddition reactions. Geometry optimizations and energy calculations were performed with the Gaussian 98 program, using the B3LYP density function and 6-31+G (d) basis set. GaussView was used to visualize the transition state structures. The theoretical calculations predicted the most stable dimer forms. The theoretical results have been confirmed by HPLC isolation experiments, which together with the UV spectra of the different products, verify the presence of the different conformers.

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PI66**The UVB filter, 2-phenylbenzimidazole-5 sulfonic acid, enhances the formation of cyclobutane pyrimidine dimers and oxidative damage following UVA and simulated sunlight irradiation***N. Bastien¹, M. Rouabhi², R. Drouin¹**¹Service of Genetics, Department of Pediatrics, Faculty of Medicine and Health Sciences, University of Sherbrooke, Sherbrooke, Quebec, Canada; ²Groupe de recherche en écologie buccale, Faculté de médecine dentaire, Université Laval, Quebec City, Quebec, Canada*

The 2-phenylbenzimidazole-5 sulfonic acid (PBSA) is widely used as a UVB filter in sunscreen and cosmetics. However, it is known that PBSA can be decomposed, and subsequently photosensitize the guanine residues after irradiation of purified oligonucleotides (Stevenson and Davies, 1999, *Chem Res Toxicol* 12:38-45). Does the PBSA have the same effect in a cellular context? We irradiated fibroblasts, keratinocytes, engineered human skin (EHS) and purified DNA with 250 kJ/m² UVA, 50 kJ/m² UVB and 4,000 kJ/m² of simulated sunlight (SSL) in presence of different PBSA concentrations ranging from 0 to 4 mM. We showed that PBSA protects cells and DNA against the formation of cyclobutane pyrimidine dimers (CPD) after UVB irradiation. This protection is less effective with EHS. After UVA irradiation, we found an increase of CPD formation with doses of PBSA in cells and EHS, but not in DNA, and also, an increase of oxidative damage with the different doses of PBSA for cells, EHS and DNA. Upon SSL irradiation, the PBSA enhances oxidative damage formation in DNA, cells and EHS, however, protecting against CPD formation, although less effectively than for UVB. These results suggest that PBSA, which can protect against UVB irradiation, can stimulate CPD and oxidative damage formation following UVA and SSL exposure. This work was supported by a grant from the National

Cancer Institute of Canada (with funds from the Canadian Cancer Society).

PI67

On the influence of application amount of sun protection products on their efficacy and photostability

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The effect of sun protection products (SPP) is determined internationally by means of standardized *in vivo* methods. One point that is criticized about these standards is the application amount of 2 mg/cm², which, compared to practical application, is too high and which turns the determined sun protective factor (SPF) into a mere "index".

Since the *in vivo* methods also have numerous disadvantages (reproducibility, test time and expenditure, ethical reservation), it has been attempted for quite some time to complement or substitute them by *in vitro* methods. The objective of the test was to compare the *in vivo* and *in vitro* sun protection factors (SPF) for commercial SPP and thus supplementing former measurements. Furthermore, the influence of different application amounts on the SPP and their photostability was to be determined *in vitro*. Application amounts of 0.75, 1.0 and 1.25 mg/cm² were examined. The SPP were divided into 4 groups with different efficacy. A change of application amount to + and – 0.25 mg/cm² led to a decrease or increase of the SPF. Halving the application amount from 1.5 mg/cm² to 0.75 mg/cm² led to a reduction of the protective effect on a scale of about 50 %. In total, photostability had a tendency to decrease with higher application amounts (in order to reach 1 MED). This becomes clear when the application amounts are halved.

Conclusion:

1. The correlation coefficient between *in vivo* and *in vitro* measurements is 0.85.
2. The necessary application amount in order to reach the same SPF is directly proportional to the roughness values of the surface.
3. An increase or decrease of the application amount by 1% leads to a proportional increase of the SPF by 1% as well.

PI68

Influence of substitution at the benzylic position on the behavior of stereoisomeric phosphorus compounds as precursors of possible antioxidants

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Antioxidants capable of blocking free radical chain processes must meet two requirements: 1) they should be good hydrogen donors and 2) the resulting radicals should exhibit low reactivity toward oxygenⁱ. One of the strategies to determine the ability of a molecule to act as antioxidant is the use of transient absorption spectroscopy to investigate the reactivity of *tert*-butoxy radicals towards H-donors. If the new radicals formed have suitable absorption bands, their formation and reactivity can be studied by the laser flash photolysis (LFP) technique.

We have previously reported that benzo[*d*]oxaphospholes can be useful as precursors of stabilized carbon-centered radicalsⁱⁱ. Interestingly, the presence of phosphorus in their structure gives rise to the formation of two diastereoisomers. This particular structural feature has allowed the study of diastereodifferentiation in the process.

We wish now to report on the results obtained with 3-alkyl-substituted derivatives, whose study was undertaken in order to understand the factors involved in the intriguing

diastereodifferentiation observed before. In addition, compounds lacking the substituent at the benzylic position, as well as the non-cyclic analogues, have been also studied to determine whether the phosphorus-containing heterocycle plays a key role. Furthermore, steady-state photolysis studies have been carried out; the main products are diastereoisomers resulting from cross-coupling between the oxaphosphole system and the solvent. These products are formed in a diastereomeric ratio that is independent from the precursor configuration, indicating that the C-centered radical does not show any memory effect.

A remarkable influence of substitution at the benzylic position on the behavior of the stereoisomeric phosphorus compounds was observed: while, in aryl-substituted derivatives the *trans*-stereoisomer showed higher efficiency of radical formation, the *cis*-isomer was more reactive in the alkyl-substituted analogues.

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PI69

Antioxidant activity of mycosporine-like amino acids (MAAs) from marine dinoflagellates

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We evaluated the effects of ultraviolet radiation (UVR, 280-400 nm) on growth and production of UV-absorbing compounds of two marine dinoflagellates: *Gymnodinium chloroformis* and *Heterocapsa triquetra*. Both species were incubated in an illuminated chamber (20°C; 66, 15, and 0.7 W m⁻² of PAR, UV-A, and UV-B, respectively) for about 10 days under two radiation treatments (PAR and PAR+UVR). There was no significant increase in the content of MAAs in *G. chloroformis*; in *H. triquetra*, however, the concentration of MAAs (mainly shinorine, determined by HPLC techniques) increased almost 4 times after 8 days of exposure to UVR, suggesting an UVR-induced accumulation. The antioxidant activity of *H. triquetra* extracts were tested by *in vitro* methods such as β-carotene linoleate model and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging. Hydroxyl radical and superoxide anion scavenging were determined by measuring competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe³⁺/ascorbate/EDTA/H₂O₂ system and by xanthine oxidase system, respectively. The extract showed inhibitory activity of β-carotene bleaching and acted as primary antioxidant, being capable of donating hydrogen to the free radicals (IC₂₅ 3,8 ng/ml), leading to non-toxic species and therefore to inhibition of the propagation phase of lipid oxidation. The extracts also showed scavenging activity of superoxide anion and hydroxyl radical, suggesting that MAAs might protect *H. triquetra* cells against oxidative stress. Active extracts of *H. triquetra* may be an alternative synthetic antioxidant that could be used as additives for nutrition, pharmaceutical and cosmetic purposes.

PI70**Seasonal changes in UV exposure***S. Wengraitis, D. Sliney**US Army Center for Health Promotion and Preventive Medicine*

A CIE Technical Report, "Spectral Weighting of Solar Ultraviolet Radiation," prepared by CIE Technical Committee TC 6-25 provides calculated estimates of biologically effective solar ultraviolet radiation (UVR) for various altitude, ozone, and solar zenith-angle conditions. The biological effects included erythema. Using the CIE report along with an astronomical solar-position program we plotted overlapping data of the solar zenith angle and hour-by-hour variations in the Global Solar UV Index at sample location, for various months of the year. Such plots would not only be a useful tool for health authorities -- by allowing users to quickly identify the conditions where risk of overexposure is the highest—but may also suggest which times of the year would be most beneficial for those seeking solar UVR exposure for health benefit, while at the same time minimizing health risk. The utility of these plots for this purpose will be investigated.

CIE Report published as CIE Document 151:2003.

PI71**Pulsed radiation studies of natural UV filters extracted from lichens: possible sunscreens***R. Edge**Keele University & CCLRC Daresbury, UK*

There is interest in compounds extracted from natural sources as active substances in medicine. Compounds extracted from lichens that survive in regions where there is ozone deficiency (Southern Chile) may act as possible sunscreens and, since none of the current commercial sunscreens are perfect, the search continues for improved formulations.

We report on lichens using laser flash photolysis and pulse radiolysis to obtain the photophysical parameters of the extracts. The compounds studied are Usnic Acid (Usn), Calycine (Cal), Boldine (Bol – extracted from Boldo trees), epiphorillic Acid I and II (Epi I and II), Viconicine (Vic) and 1-Chloropannarin (Clp).

Laser studies showed low triplet and singlet oxygen yields (<0.01), essential for sunscreens. However, Epi I and II and Vic exhibited high triplet energy levels so there is a danger of sensitising target molecules. Singlet oxygen quenching rate constants of the others will also be reported.

Usn and Cal were found via pulse radiolysis to be efficient quenchers of free radicals. Such quenching leads to quencher radicals themselves being generated (e.g., $RO_2^* + \text{Lichen} \rightarrow RO_2^- + \text{Lichen}^*$) and the consequences of this must be considered (true of all antioxidants and often ignored). We have shown that ascorbic acid can repair such Lichen radicals, suggesting improved efficiency if used with vitamin C.

The consequences of our results for all the natural compounds studied as potential sunscreens for future (biological) studies will be discussed.

I thank Drs S. Navaratnam, F. Rancan and Profs E Fernández, F Boehm and G Truscott for collaboration. Experiments were carried out at the FRRF, Daresbury Laboratory, Warrington, supported by EU "Improving Human Potential" Transnational Access to major Research Infrastructures (Contract HPRI-CT-2002-00183).

PI72**Betamethasone phototoxicity: *in vitro*, *in vivo* and *ex vivo* studies***G. Miolo¹, F. Gallochio¹, S. Caffieri¹, F. Baccichetti¹,**C. Marzano¹, M.G. Zanirato², G. Beyersbergen van Henegouwen¹**¹Dipartimento di Scienze Farmaceutiche, Università di Padova,**Italy; ²Dipartimento di Prevenzione-Servizio Veterinario, ASL 17, Conselve, Italy*

Betamethasone is a synthetic corticosteroid present in numerous systemic and topical commercial formulations for the treatment of rheumatic diseases and skin disorders. The drug is highly unstable

under UV light (UVB>UVA) and it has demonstrated moderate photosensitizing activity in patients exposed to solar radiation. Since this drug is taken to treat skin diseases, it's likely that its induced photosensitization reactions are not always recognized as they are taken for mild sunburn or solar eczemas as well. Three methods (*in vitro*, *in vivo* and *ex vivo*) were used to test drug phototoxicity. The *in vitro* phototoxicity was determined by exposing Balb/c 3T3 fibroblasts to UVB radiation in the presence of 100 μM drug. The results showed that dark incubation and low UVB dose (0.4 J/cm^2) did not affect cell viability. On the contrary, upon exposure to this low UVB dose, Betamethasone was able to induce a decrease of cell viability (30%).

In vivo phototoxicity was assayed by means of skin erythema induction in albino guinea pig skin. The drug (200 μg) induced skin erythema both when applied as methanol solution (+/-) and as commercial formulation (cream containing 0.1% Betamethasone) (+/+/-).

An *ex vivo* model, epidermis and dermis from isolated pig skin, was also employed. By working both with intact skin and with isolated epidermal cells, it has been possible to determine the extent of intact drug recovery and the cell viability after drug phototreatment. The three methods used gave comparable results and therefore *in vitro* and *ex vivo* tests may be an alternative to *in vivo* phototoxicity studies.

From our results, Betamethasone can be considered a photosensitizing drug.

PI73**Effects of aqueous preparations on the phototoxicity of curcumin***E.M. Bruzell¹, E. Morisbak¹, H.H. Tønnesen²**¹Scandinavian Institute of Dental Materials (NIOM), P.O.Box 70,**N-1305 Haslum, Norway; ²School of Pharmacy, University of Oslo,**P.O.Box 1068 Blindern, N-0316 Oslo, Norway*

Curcumin (CU) may be a potential photosensitizer for oral applications. An ongoing study evaluates the bactericidal effects of CU in five aqueous preparations. The present work investigates photosensitizing effects of CU in these preparations in oral, non-cancerous cells. CU is a natural food colorant with absorption peak at 430 nm. In its ground state, CU has shown potential tumour preventing activity in several cancer cell lines, but apoptosis is seldom induced in normal or primary cells. The phototoxic effect of CU has not been thoroughly investigated. It is a challenge to make an aqueous CU preparation with an acceptable solubility and stability at physiological pH. Salivary gland acinar cells were exposed to CU in DMSO, Triton X-100 (TX), cyclodextrin (CD), liposomes (LP) or an alginate solution, and irradiated with a halogen lamp emitting mainly blue light. Changes in enzyme activity were determined by MTT test, and cell death by staining-/microscopy- and Western immunoblotting techniques. Irradiation of cells exposed to 13.5 μM CU in DMSO or TX in tissue culture wells with 1.6 J/cm^2 reduced enzyme activity to about one third compared with dark controls. However, increasing the light dose to 2.7 J/cm^2 did not decrease enzyme activity accordingly. A concentration of 0.7 μM CU did not reduce enzyme activity compared to controls in any of the preparations irrespective of irradiation. No decrease in enzyme activity was observed when irradiating cells exposed to CU in CD, LP or alginate irrespective of concentration. Exposure of cells in dishes to 13.5 μM CU in CD and 6 J/cm^2 increased cell death five-fold compared to dark controls and 10-fold compared to vehicle controls. When cells were exposed to 0.4 μM CU in liposomes and 6 J/cm^2 , cell death increased 10-fold compared to dark controls. The liposome preparation was the most efficient vehicle to induce cell death. A careful selection of the vehicle is of importance when applying curcumin as a sensitizer.

PI74**Singlet excited state properties of fluoroquinolones: emission of norfloxacin and derivatives in aqueous media**

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The photophysical properties of some norfloxacin (NFX) derivatives have been studied in order to evaluate the role of the free carboxylic acid and the non-protonated piperazinyl group. Absorption, emission and excitation spectra of the compounds were recorded at different pHs. The results have provided clear evidence in favour of singlet excited state deactivation of NFX and pefloxacin (PFX) via intramolecular electron transfer from the N(4') atom of the piperazinyl ring to the FQ main system. This is a very efficient, energy-wasting pathway, that becomes dramatically enhanced in basic media. Acetylation at N(4'), to give ANFX, decreases the availability of the lone pair, making fluorescence observable even at high pH. It also reveals that the geometry of FQs changes from twisted (ground state) to nearly planar (singlet excited state); accordingly, the singlet energy of ANFX is significantly lower than that of NFX and PFX. Finally, further experimental evidence is provided confirming the previously proposed role of intramolecular hydrogen bonding between the non-ionised carboxylic acid and the 4-oxo group, as well as the nature of static/dynamic fluorescence quenching by phosphate anions.

PI75**Photophysical techniques for the study of drug-protein interactions: flurbiprofen-human serum albumin as model system**

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Drug-HSA interactions play a key role in the control of drug biodistribution, metabolism, elimination and pharmacological effect in the body.

Here we wish to report on the suitability of the lowest triplet excited states of drugs as probes to investigate the important issue of drug binding to transport proteins. As their properties are very sensitive to the experienced microenvironment, these states should allow to study the drug distribution among the bulk solution and the different protein binding sites, providing valuable information on the nature of the drug-protein complexes (i. e., strength of the interaction, conformational restrictions, protection from attack by oxygen or other reagents, stereochemical requirements...). The triplet lifetimes of (*S*)- and (*R*)-flurbiprofen methyl ester (FBPMe) in the presence of HSA have allowed to distinguish between the two HSA binding sites (site I and site II). The occupation level of these binding sites has been estimated from regression analysis of the triplet decays at several [FBPMe]/[HSA] ratios. Besides, a remarkable stereodifferentiation has been found in the triplet lifetimes within the protein microenvironment.

To understand the above results, bichromophoric compounds containing flurbiprofen and the relevant amino acids present in the binding sites (Tyr and Trp) have been prepared and studied by fluorescence and laser flash photolysis. Thus, the involved mechanisms have been established in well-defined chemical systems, where the non-covalent supramolecular drug-protein interactions are modelled by the analogous intramolecular processes in covalently linked dyads.

PI76**Stereodifferentiation in the interaction between chiral carprofen and Human Serum Albumin : from whole protein to model dyads**

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In the last decade, asymmetric photochemistry has attracted a lot of interest. This area of chemistry can be of special interest for the photobiologist when it involves drugs and biomolecules. In this context, possible stereoselective photoprocesses during biomolecules sensitization would inform about the most photoactive stereoisomeric drug and provide additional data to consider in the switching towards pure enantiomers. In spite of the significance of such a study to improve the benefit to risk ratio, the photobiological properties of chiral drugs remain practically unexplored.

In this work, we describe the interaction between chiral carprofen (CP, a NSAID largely reported for its photosensitizing properties) and Human Serum Albumin (HSA), or tryptophan as relevant amino acid.

In a first step, Laser Flash Photolysis experiments have revealed a stereodifferentiation in the lifetime of CP triplet state when complexed with the protein. This result has been associated with stereoselective formation of the dechlorinated photoproduct but also with the formation of covalent CP-HSA photoadducts. In both cases, tryptophan has been proposed to be the relevant amino acid.

To verify this hypothesis, model carprofen-tryptophan dyads have been synthesized and studied. Analysis of the photoproducts as well as triplet state stereodifferentiation allowed to confirm the involvement of tryptophan and to conclude to a photoredox mechanism between the excited drug and the amino acid.

PI77**Singlet oxygen-mediated photodegradation of folic acid and photosensitizing activity of its photoproducts**P. Vorobey^{1,2}, M.K. Off¹, A. Vorobey^{1,3}, J. Moan¹

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Folic acid (FA) is a synthetic compound widely used as vitamin supplement for prevention of several diseases. We have shown that FA in aqueous solution can easily be degraded by singlet oxygen. Singlet oxygen was generated using water-soluble porphyrins (TPPS₃ or TPPS₄) and blue light exposure. The rate of porphyrin photosensitized degradation of FA drastically increases in heavy water. This fact and the inhibition of the action by sodium azide confirm the key role of singlet oxygen in the process. FA is also highly photosensitive itself, and can be degraded by UV-A radiation with formation of p-aminobenzoyl-L-glutamic acid and two fluorescent pterines – 6-formylpterin (FPT) and pterin-6-carboxylic acid (PCA). Kinetics of UV-A induced FA degradation in air-equilibrated aqueous solution obeys an exponential law. Because FPT and PCA can produce singlet oxygen when exposed to UV-A radiation, we believe that the kinetics may be determined by the ability of FA photoproducts to generate singlet oxygen and in this way sensitize the photodegradation of FA. In oxygen-free solution the rate of FA photodegradation is much lower than in the presence of oxygen, and the kinetics of the process is linear. UV-A induced FA degradation is inhibited by sodium azide. These findings indicate that singlet oxygen is an important agent in direct FA photodegradation. The sensitivity of FA to singlet oxygen and photosensitizing activity of its photoproducts may pose hazards to biological systems. We observed the degradation of indole rings of tryptophan and tryptophanyles in human serum albumin upon

UV-A exposure of solutions containing FA. In suspension of erythrocytes with inclusion of FA and exposure to UV-A radiation or to sunlight the formation of fluorescent FA photoproducts and the induction of hemolysis are observed. This work was supported by The Belarusian Republican Foundation for Fundamental Research and by The Research Foundation of The Norwegian Radium Hospital.

PI78**Effects of buffers, pH, and hydroxylated molecules on fluorescence emission of protein**

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Measurements of micro quantities of proteins is essential in reactions and isolates. Although spectrophotometry has traditionally been used for measuring protein concentrations, there is a need to adopt alternative measuring methods. In this study, bovine serum albumin (BSA) was used as a standard protein and its concentration is monitored by fluorescence spectrometry at the excitation wavelength of 300nm and emission at 345nm using different solution conditions. Three different buffers, which are commonly used in biochemical reaction, were tested for their effects on the fluorescence intensity. The buffers tested, phosphate, tris, and hepes, showed enhancement of intensities about 3 folds relative to water solution. Elevation of fluorescence intensities was also seen with increasing pH conditions. By setting a suitable solution conditions, a fluorophore-like fluorescent emission can be established by the protein itself. An indication of hydrogen interaction disruption and relocation was observed as the use of hydroxylated molecules, ethanol and phenol, caused a decrease in the intensities. Furthermore, a comparison of standard curve of intensity fluorometry to the absorbance spectrometry at different BSA concentrations was tabulated.

PI79**Photosensitizing activity of di- or tetraaryl-porphyrins on HCT116 cells**

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In photodynamic therapy (PDT), molecular oxygen, light and a photosensitising agent are used in combination to kill cancer cells. Both totally synthetic photosensitizers and agents prepared from natural compounds *via* simple synthetic procedures have been the object of intense studies in the last decade. The efficacy of the treatment is strictly related to the drug photophysical and chemical properties; about the latter properties it is generally accepted that photosensitizers featuring two different sites, hydrophobic and hydrophilic moieties, display improved tumour specificity. Up to date a few molecules have received clinical approval then the synthesis and characterization of novel photosensitizers could yield drugs with better properties i.e. an improved tumour tissue retention and/or higher normal tissue/cancer cells selectivity, high quantum yield of reactive oxygen species, rapid clearance from normal tissues.

In this study we evaluated the photo-toxic activity of a number of new, non symmetric *meso* di- and tetraaryl substituted porphyrins. Their effects were assessed *in vitro* on the human colon adenocarcinoma cell line (HCT116) and the results compared with those obtained with Photofrin and with the tetra-(*m*-hydroxyphenyl)-chlorine, both approved for clinical use.

Cytotoxicity studies, performed by MTT assay following 24h exposure to the compounds, 2h irradiation with a 500 W tungsten-halogen lamp (water filter used) and 24h in drug-free medium, showed that some of the 5,15-diaryl compounds are more effective than both 5,10,15,20-tetraarylporphyrins and reference compounds. None of the compounds tested exhibited significant toxicity in the

dark. Flow cytometric analysis of HCT116 cells treated with the more active compounds in the panel showed a relevant increase in apoptotic cell death; this effect was associated with a significant increase in reactive oxygen species (ROS) levels and cell cycle alterations.

PI80***In vitro* photodynamic efficacy of emodine bearing porphyrins**

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Hypericin is a natural organic compound whose photosensitising potential has been known for years, although it is known that has a poor solubility under physiological conditions and low maximum of absorbance in the visible region above 400 nm; furthermore the chemical modification of its structure can be hardly achieved. An Hypericin precursor, Emodine, has a higher availability and can undergoes to chemical modifications leading to the introduction of functional groups on its periphery. The presence of an aldehyde, carboxylic acid or bromo-methyl group on the Emodine skeleton allows the design of porphyrins bearing Emodine either as *meso* phenyl peripheral substituents or covalently attached onto *meso* positions. Following different synthetic approaches we have synthesized a series of new photosensitizers in which both tetrapyrrolic skeleton and one Emodine unit are present.

The photodynamic efficacy of the new compounds were assessed *in vitro* on the human colon adenocarcinoma cell line (HCT116) in order to investigate a possible synergistic or a merely additive effect of the two photosynthetic systems. Cytotoxicity studies were performed by MTT assay following 24h exposure to the compounds, 2h irradiation with a 500 W tungsten-halogen lamp (water filter used) and 24h in drug-free medium. The results are expressed as IC₅₀ values and compared with those obtained with Photofrin and with the tetrakis-(*m*-hydroxyphenyl)-chlorine.

These new Porphyrin-Emodine photosensitizers do not show a good efficacy, however few indications allow to believe that simple structural modifications can increase the activity; in particular it was shown that that photosensitising action is strictly correlated to the presence of protected or non protected hydroxyl groups.

Some synthetic approach to hypericine bonded porphyrins will be also discussed.

Parts of our research were supported by Indena founding.

PI81**Photostabilities of some photoactive organic species common in some sunscreens with different SPF**

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The presentation is focused on the results of the photostability studies of some photoactive agents common in commercially available sunscreens in solvents of varying polarities from water (to mimic water in oil w/o emulsion) to least polar n-hexane (to mimic the oil in water emulsion o/w) that actually used in sunscreen formulations.

Attempts have been tested to stabilization of some active agents in presence of different combinations of chemical filters and/or physical filters such as TiO₂ (microparticles), TiO₂ (Degussa, nanoparticles) or ZnO. Neither complex combinations of organic filters nor addition of inorganic filters could absolutely prevent photodegradation or the formation of less efficient UV absorber such as the *Z*-isomer in case of cinnamate or the camphor derivatives. The results indicated that some of the photoactive organic species commonly used today in sunscreens are unstable following irradiation.

Furthermore, some commercially available sunscreen lotions with different sun protection factors (SPF's) showed changes in absorption spectra upon exposure to UV/Visible irradiation leading to reduction of the expected photoprotection of human skin and DNA against the harmful light radiation. Photodegradation has been detected and leads to loss in light absorption in UVA and UVB regions concomitant with formation of new photoproducts that absorb in the UVC region.

Finally, open questions regarding photodegradation products, limited protection and the effect on human skin will be addressed.

PII1

Enhanced selectivity of tri-component pro-drugs: enzyme specificity

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It is possible to enhance the loading of a drug in a particular tissue site, such as in the vicinity of tumour tissue, by use of the specific characteristics of the tumour. Tumours often over-express certain types of highly specific proteolytic enzymes that break down the structural protein matrix of the surrounding normal tissue. We have thus designed a series of pro-drugs that make use of this enzyme specificity in order to enhance drug delivery at the tumour invading front. These pro-drugs contain three components: an active agent (either a chemo- or a photochemotherapeutic type of agent), a solubilizer (such as glucosamine or tetraarginine), and a polypeptide chain linker that is recognized by the specific proteolytic enzymes that are present at the surface of invading tumours. Two drugs were used in this study. The first drug was susceptible to cleavage by the protease chymotrypsin and the second was susceptible to cleavage by a proteolytic enzyme which is over-expressed by tumours, a matrix metalloproteinase. The first drug consisted of hematoporphyrin IX (HpIX) linked to a tetraarginine solubilizer via the polypeptide sequence Val-Val-Phe. This linkage can be cleaved at the Val-Phe linkage by chymotrypsin. The second drug consisted of a pyropheophorbide linked to a solubilizing glucosamine via the polypeptide sequence Pro-Gly-Leu-Pro-Gly. This linkage can be cleaved at the Leu-Pro linkage by matrix metalloproteinase-2 (MMP-2). Both pro-drugs were exposed to two types of enzymes in order to test the non-reactivity and reactivity of the specific linkages. These results verified that an appropriate peptide linker could be designed in order to achieve selective delivery of appropriate active agents.

PII2

Aggregation susceptibility on phototransformations of hematoporphyrin derivatives

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PDT relies on the use of light sensitive compounds, which selectively locate in tumor tissues. Upon excitation of the sensitizer with light of the appropriate wavelength, reactive oxygen species are formed that are able to inactivate the tumor cells. Most of the photosensitizers used in PDT suffer degradation by light. In this work, photobleaching of Photogem[®] (PG), Photofrin[®] (PF) and Photosan[®]-3 (PS), hematoporphyrin derivatives produced in Russia, Canada and Germany, respectively, was induced by LED (630nm, 25mW/cm², 360 min) in the presence or absence of 1% Triton X-100, a non-ionic detergent used to mimic the membrane hydrophobic environment. Photobleaching and photoproducts formation were monitored by fluorescence and absorption. The kinetics parameters were obtained fitting the data using the first order equations. For both, degradation (Soret band) and photoproduct formation, Triton showed a strong effect for PF and PS (τ of degradation decreased around 50%) while for PG only 8%,

in agreement with the increase of the quantum yield of fluorescence (50x for PS, 16x for PF and 6x for PG) due to the presence of detergent. The efficiency of photoproduct formation, which was accessed by $\Delta A_p/A_{630}$, where A_p is the photoproducts absorbance at 640 or 660nm and A_{630} is the absorbance at the illumination wavelength, increases in the presence of Triton X-100. Again, PS showed to be the more sensitive (efficiency in 640 nm increases by 15x) but the photoproduct accumulation with PG was inhibited in the presence of Triton (decreases by 50%). The 660nm band has not been used to measure the Triton effect since this band does not exist without detergent. The Triton effect should be related to aggregation susceptibility, since the fluorescence, degradation and photoproduct formation are affected. The higher aggregation attributed to PS in water, should be due to lowest content of monomers followed by PF and PG.

PII3

In situ detection of singlet oxygen in intact HT29 cells by MALDI-TOF mass spectrometry

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Photodynamic therapy (PDT) is applied for palliative treatment of head and neck cancers and several forms of skin cancers. A photosensitizer can induce a cytotoxic activity and microvascular damages notably due to the production of reactive oxygen species such as singlet oxygen (¹O₂). The purpose of our studies has been to detect singlet oxygen in intact cells generated by a photosensitizer 5,10,15,20-tetrakis(m-hydroxyphenyl) chlorin (m-THPC, Foscan[®]) which is a potent second generation photosensitizer. These studies consist of the understanding of PDT mechanisms on colo HT29 cells by MALDI-TOF Mass Spectrometry. Firstly, we have developed a new sampling protocol which allows a direct analysis of the intact HT29 cells by MALDI-TOF-MS. Like this, we can detect by MALDI-TOF/MS the ortho-benzoilbenzene (o-BB) which is generated by the reaction between singlet oxygen and the molecular probe 1,3-DiPhenylisoBenzoFurane (1,3-DPBF). On other hand, the first results concerning the use of a another probe (trans-1-(2'-methoxyvinyl)pyrene) will be shown. This technique allowed us to show on the same spectra: the photosensitizer, the singlet oxygen acceptor and the cell pattern. An important step is to prove that the molecular probe DPBF is inside the intact cells. For answering this question, we used a protocol on the permeability of the cell membrane.

Secondly, we have made studies concerning the appearance or disappearance of such proteins. These experiments should be compared with proteomic approaches to define their sequence. These studies allow us to show the potentiality of the in situ MALDI-TOF-MS analyses compared to the classic electrophoretic method used in proteomic research.

PII4

On the correlation between hydrophobicity, liposome binding and cellular uptake of porphyrin sensitizers

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Porphyrins and their analogues are used as photosensitizers in photodynamic therapy. A crucial factor in choosing a photosensitizer for PDT is its ability to incorporate into the cells. For hydrophobic compounds that partition passively into the cytoplasmic membrane, a partition coefficient between an organic solvent and water, P , is one factor that could be used to predict the molecule's ability to diffuse into biomembranes. We synthesized several analogous porphyrins, some with structures that are closely related to *m*-THPC. The porphyrins have the tetrapyrrole ring core, with 2, 3 or 4 *meso* functionalization substitutions. We studied the

spectroscopic and photophysical properties of these porphyrins, and found their singlet oxygen production yields. We calculated their theoretical octanol:water partitioning, namely $\log P$, as a parameter of hydrophobicity. We measured fluorimetrically their partitioning constants into liposomes (K_b) as well as their uptake by cells. We then examined the correlation between $\log P$ and K_b and the uptake by cells. An average of several commercial softwares that predict $\log P$ shows that these porphyrins are very hydrophobic, with $\log P$ values in the range 7.4-10.4. The correlation between the estimated $\log P$ and K_b is nearly linear with a negative slope, indicating apparently that there is lesser binding to liposomes with increased hydrophobicity. All the studied porphyrins are taken up by cells, but there is no clear correlation between cellular uptake and the estimated $\log P$ or measured K_b . Lipinski's pharmacological rule of 5 predicts that poor absorption or permeation of drugs into cells is more likely when, among five criteria, a drug has $\log P$ greater than 5. This may be relevant with liposomes' diffusional binding, where aqueous aggregation can interfere strongly, but may not be the case with cellular active uptake. Thus, in such extreme conditions, neither liposome binding nor other rules seem to predict the porphyrins' behavior *in vitro*.

PII5

The thermodynamic effect of temperature and ionic strength on the binding of porphyrins to liposomes

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We studied the effect of solution electrolyte on the binding of hematoporphyrin (HP) to lecithin liposomes. The binding process was monitored by the fluorimetric spectral changes that occur upon titration of HP with liposomes. Titrations were carried out at different concentrations of KCl. Singular Value Decomposition analysis was employed to resolve the measured spectra into the contributions of the lipid-bound and the free aqueous HP, in order to obtain their equilibrium concentrations. The binding constants to lecithin liposomes that were thus obtained increase from 2.5 (mg/ml)⁻¹ in water to 5.5 (mg/ml)⁻¹ at 0.4 M KCl. Higher concentrations did not demonstrate any further change in the binding constant. Thus, a salting-out of HP from the aqueous phase into the lipid environment is evident.

We also set-out to find whether the temperature, which affects chemical kinetics and equilibria, also influences the rate and extent of the binding of HP to lipid membranes. We used the technique that was described earlier, to measure the effect of temperature on the binding constants of HP to liposomes composed of lecithin or DMPC. The binding process was almost instantaneous above the phase transition temperature but it was very slow below this temperature. Thus, the close packing of lipid molecules in the solid-gel phase slows down the diffusion into the membrane. A very distinct effect of the phase transition temperature on the binding was observed. In DMPC the binding decreased from 16 (mg/ml)⁻¹ to 4.2 (mg/ml)⁻¹ while the temperature was increased from 11°C to 27°C, respectively. When the temperature was further increased above the solid-to-liquid phase transition temperature, from 27°C to 37°C, the binding constant increased at a much smaller rate, from 4.2 to 7.0 (mg/ml)⁻¹.

PII6

Acid-base properties and liposome binding of a perfluoroalkylated phthalocyanine

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We synthesized a perfluoroalkylated derivative of phthalocyanine, and studied it spectroscopically and photophysically, as the free base, F₆₄PcH₂, as well as a zinc complex, F₆₄PcZn. The perfluoroalkylation of the phthalocyanine core is expected to enhance the lability of the core hydrogen atoms, which should

become more acidic. The spectroscopically monitored pH titration of the free-base show that F₆₄PcH₂ undergoes unique basic and acidic proton exchange processes whose spectroscopic signatures are explained on the basis of the chromophore's symmetry. Singular Value Decomposition (SVD) analysis resolves the spectra into the contributions of the individual species that co-exist at various stages of protonation and reveals the associated equilibrium constants. Only two species exist in the pH range 3-11, with a pK around 6, namely, the deprotonated (F₆₄Pc⁻²) and the neutral (F₆₄PcH₂) forms. We also measured the effect of pH on the binding of the free-base molecule to liposomes. Surprisingly, the negatively charged form binds strongly, with a binding constant of 25 (mg/ml)⁻¹, while the neutral form exhibits immeasurably weak binding. Both species exhibit photosensitizing properties and generate singlet oxygen. The absolute quantum yields for metal-free negative form, F₆₄Pc⁻², was 0.252 in MeOH and 0.014 in liposomes. The zinc complex, F₆₄PcZn, had higher yields, 0.613 in MeOH and 0.12 in liposomes.

PII7

Microscopic studies of cellular uptake and photocytotoxicity of hematoporphyrins in cancer cells. Effect of pH on the affinity, penetration depths and sensitization in membranes

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Photosensitization by porphyrins and other tetrapyrrole chromophores is used in biology and medicine to kill cells. This photosensitized damage that is caused to the host cell occurs in competition with the rapid diffusion of singlet oxygen through the lipid phase and its escape into the aqueous phase. In this study we showed that the extent of damage can be modulated by employing modified hematoporphyrin (HP) analogs, which have chemical "spacers" of varying length between the tetrapyrrole part of the molecule and a carboxylate moiety. These derivatives have essentially the same chemical attributes and reactivity as the parent compound, HP IX, which is used in clinical procedures of photodynamic therapy. We demonstrated that increasing the number of methylenes in the carboxylate chain increases the binding of the HP derivatives to the liposomes. We also showed that the pH affects the extent of partitioning of these HPs into membranes, through its effect on the fraction of the neutral species, which is capable of intercalating into the lipid bilayer. More importantly, pH affects the location of the porphyrin molecule in the membrane and the neutral species, obtained upon lowering the pH, is embedded at a deeper location in the lipid bilayer. This, in turn, has a strong effect on the efficiency of photosensitizing an oxidation reaction to a membrane-bound singlet oxygen target. Further decrease in pH generates protonation of the pyrroles and a reverse of the above effect. The practical implication is that both a non-chromophoric modification of the sensitizer and a change in pH control the vertical displacement of a sensitizer in a membrane and amplify the observed efficiency of photosensitization. Fluorescence microscopy verifies the rapid uptake of hematoporphyrin analogs to cancer cells and images received by scanning electron microscopy demonstrate the strong damage caused to the membranes of photosensitized cells.

PII8

Modularly synthesized porphyrazines with tuned hydrophobicity for near IR photosensitization

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Three novel modified porphyrin-like structures were synthesized to form modular structures in which the lipophilicity and water

solubility can be tuned. The general structure takes the form $H_2[pz(A_nB_{4-n})]$, where the core is a porphyrazine (pz) group, **A** is $[S(CH_2)_3COOR]_2$ ($R = n\text{-Pr, H}$) and **B** is a fused β,β' -diisopropoxybenzo group, with $n = 4, 3,$ and 2 . These molecules possess absorption bands at between 700 nm and 810 nm, so laser beams of higher tissue penetration depth could be used to illuminate them. Armed with absorption bands in the far-red and near-IR, and a capability to tune the solubility, these molecules could make for better sensitizers due to optimized uptake by lipidic membranes and better optical properties. We tested several derivatives of the A_4 , A_3B , and A_2B_2 structures for their singlet oxygen quantum yields in liposomes and in methanol, using dimethyl anthracene as a singlet oxygen target. Singlet oxygen quantum yields in liposomes ranged from 0.01 to 0.44, with the A_2B_2 group showing the most promise. In the binding assay to find the equilibrium binding constant, K_b , we detected fluorescence changes due to a change in environment. Peripheral long-chain moieties dominate lipid binding more than the core. These moieties range in the hydrophobicity that they induce from C_8H_{17} and benzene, which rendered the molecule totally insoluble in water, to polyethylene glycol and carboxylate groups, which imparted water solubility. Each molecule had between 4 and 8 identical chains. Chains bearing an ether or ester link gave us measurable equilibrium constants, with a higher K_b for ether substituents. Results for K_b ranged from 1 to 76 (mg/ml)^{-1} . A delicate balance exists between water solubility, and good partitioning to membranes. In general, higher oxygen-to-carbon ratio in the chains improves binding. Fewer chains, and a centrally coordinated metal ion further improve binding and singlet oxygen production.

PII9

Interaction between porphyrins and filamentous phages as non-covalent supramolecular antenna system

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Well-defined arrays of chromophores are of great interest not only as mimics of natural antenna systems but also because they can display unique optical and physical properties. The key issue is to incorporate a large number of chromophores with known photophysical properties into a precise arrangement over the entire nanosized architecture. Porphyrins are widely used in assemblies of donor-acceptor materials in molecular electronics and photovoltaic devices. In the past decade, there has been a growing interest in the use of viruses as scaffolds for nanosized materials. Since certain viruses can be obtained in large quantities and manipulated at genetic level, they represent a unique opportunity for chemists to expand the collection of natural starting materials for such applications. The supramolecular approach here described is based on the electrostatic interactions between positively charged porphyrins and the coat proteins of filamentous phage (M13 or its derivatives), achieving arrays of chromophores with different levels of organization.

A variety of spectroscopic techniques have been exploited to probe interaction of porphyrins on filamentous phage surface as a function of relative concentration ratios. On titrating the *trans*-bis(N-methylpyridinium-4-yl)diphenylporphyrin (*trans*-H2Pagg) with M13 and two different M13 mutants, the results indicate the occurrence of an early stage in which small clusters of porphyrin are formed and successively the dispersion of porphyrin on the virus particle surface. In particular, circular dichroism measurements show the presence of an induced signal in the porphyrin region, as a clear evidence of interaction between the chromophore and the virus scaffold and the resulting chiral organization. The occurrence of a partial quenching of the emission of tryptophan residues present on the protein capsids together with a simultaneous enhance of porphyrin emission (exciting in the

tryptophan absorption region) point to energy transfer between this aminoacid and the interacting porphyrin. A strong dependence of the energy transfer on the disposition and number of tryptophan residues present on the surface of the different mutants has been proved.

PII10

Chiral recognition in bimolecular electron transfer between amino acids and photoactivated acceptors

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It is known that intramolecular electron transfer (intra-ET) between tyrosine (Tyr) and oxidized tryptophan (Trp) occurs in native biological reactions.ⁱ These amino acids are photooxidized by non-steroidal antiinflammatory compounds like suprofen (SUP) producing a photoallergic response due to the drug-protein photobinding.ⁱⁱ Experimental and theoretical calculations (DFT) of the photoreaction between 2-benzoyltiophene, SUP chromophore, and phenol or indole support the formation of an encounter complexes, which involve the (π,π^*) excited triple state of the ketone and the H-donor.ⁱⁱⁱ

The involvement of encounter complexes is an essential condition to observed asymmetry in the emission quenching or photosensitization. Such observation could be limited either to its formation or decay, but it could also occur in both steps. We explored the possible stereodifferentiation in the formation and decay of encounter complexes arising from the intermolecular quenching of triplet excited states of chiral suprofen methyl ester by Tyr and Trp. The determination of the pre-equilibrium constant (K_{EC}) and the intrinsic decay rate constant (k_d) for each pair indicated a significant stereodifferentiation (up to 1.5) in both steps of the quenching process, which dependent on the solvent properties.

We also considered interesting to study the capability of SUP as a photosensitizer for the intra-ET reactions in Tyr-Trp peptides as simple models of proteins. Considering the potential importance of drug chirality in biological processes, the influence of the photosensitizer configuration was evaluated. An interesting chiral recognition was observed in which the concentration of the radicals formed after triplet quenching depends on the photosensitizer configuration. Comparative studies with natural amino acids show a decreased, though parallel chiral recognition. These data agree with the involvement of an encounter complex between SUP and Tyr-Trp peptides.

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PIII1

Simultaneous determination of physical (k_q) and chemical (k_r) rate constants for singlet oxygen quenching using a steady-state IR luminescence techniqueC. Pierlot¹, J. Marko¹, J. Barbillat², J.-M. Aubry¹¹L.C.O.M., Equipe «Oxydation et Formulation», ESA CNRS 8009, ENSCL, BP 108, F-59 652 Villeneuve d'Ascq Cedex, France;²L.A.S.I.R., UMR CNRS 8516A, Bât C, F-59 655 Villeneuve d'Ascq Cedex, France

Scavenging of 1O_2 by chemical molecules includes physical quenching (k_q), in which the excited state of oxygen is deactivated without light emission, and chemical quenching (k_r) which results in the formation of various oxidation products. The overall rate constant ($k_r + k_q$) can be readily obtained by flash photolysis.

Actually two methods have been used to separate k_r from $k_r + k_q$, either by competition with a reference substrate, or by using photochemical or chemical calibrated sources of singlet oxygen. But, in all the cases the disappearance of the substrate or the appearance of the formed product has to be monitored.

We describe a new simple method for the simultaneous determination of the overall ($k_r + k_q$) and the chemical (k_r) rate constants for singlet oxygen quenching by water-soluble and organic compounds. This method uses the principles of the actual techniques for the measurements of $k_r + k_q$ and k_r respectively. The detection of singlet oxygen was achieved by its luminescence at 1270 nm as for the flash photolysis and among the photochemical and chemical sources of 1O_2 we have selected the readily available catalytic system H_2O_2/MoO_4^{2-} that is able to produce high and known quantity of singlet oxygen.

PIII2

Accumulation of sensitizer in rat embryos: spectroscopic studiesV. Legenis¹, A. Sukackaitė², V. Žalgevičienė³, G. Graželiene², J. Didžiapetriene², R. Rotomskis^{1,2}

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Photodynamic tumor therapy (PDT) is a clinically applied treatment modality of various malignant tumors based on the administration of exogenous photosensitizer, which selectively accumulates in tumours. Photoactivation of photosensitizer induces cytotoxic reactions, which cause tumor necrosis. A systemic effect of PDT on the embryos is still not known. The aim of this study was to evaluate the accumulation of Photofrin[®] in the embryos of rats at different stages of embryogenesis. The pregnant rats were administered Photofrin[®] (QLT PhotoTherapeutics (Vancouver, Canada), 5 mg/kg) into the vein of the tail on the different stages of embryogenesis. The rats were sacrificed 24 hours after the injection of Photofrin[®]. The embryos, placenta, uterus and some other rats organs were taken and examined spectroscopically. The fluorescence spectra of the embryos, as well as the excised organs, were recorded by Ocean Optics Inc. fiber spectrofluorimeter S2000-FL (a blue LED ($\lambda_{max}=400$ nm) was used for the excitation). Our results reveal that the accumulation of Photofrin[®] in the embryos depended on the stage of embryogenesis. The intensive fluorescence signal (comparable to that in the uterus) of Photofrin[®] was detected in the embryos on the 7th day of embryogenesis (ED). On the 14th ED the level of Photofrin[®] accumulation in the embryos was prominently lower than that in the other organs. On the 16th and 18th ED the level fluorescence of Photofrin[®] was lower than that on the 14th ED. On the 20th ED the fluorescence of Photofrin[®] was negligible in the embryos. The level of Photofrin[®] uptake in the embryos was much lower than that in placenta. From the obtained data it could be concluded that the accumulation of Photofrin[®] in the embryos is related to the formation of placenta and depends on the penetration peculiarities of placenta barrier. It seems that placenta prevents the penetration of Photofrin[®] into the embryo.

PIII3

Time-dependent self-assembly of 31-epimerically pure and mixed zinc methyl bacteriopheophorbides-dT. Miyatake¹, K. Shitasue¹, Y. Omori¹, K. Nakagawa¹, M. Fujiwara¹, T. Matsushita¹, H. Tamiaki²¹Department of Materials Chemistry, Faculty of Science and Technology, Ryukoku University, Otsu, Shiga 520-2194, Japan;²Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan

Self-aggregates of specific chlorophylls are found in the main light-harvesting antenna system of green photosynthetic bacteria, called a chlorosome. A number of bacteriochlorophyll(BChl)s-*c*, *d* and *e* molecules formed *J*-aggregates without any assistance of proteins. Interestingly, isolated BChls-*c*, *d* and *e* formed chlorosome-type *J*-aggregates *in vitro*, which provide a simple structural model for chlorosomes. Although many model studies for chlorosomal aggregates have been carried out, the precise supramolecular structures of the aggregates have not been clarified yet and are still debated. Studying the self-aggregation processes of chlorosomal BChls is also of great interest, because it will help us to elucidate how a chlorosomal antenna system is formed in a green bacterium. The structural diversity of the chlorosomal chlorophylls is also an interesting topic. Naturally occurring BChls take both *R* and *S* configurations as the 3¹-stereochemistry. *In vitro* aggregates of BChls and their zinc analogues showed that their supramolecular structures were dependent on the stereoisomers.

In the present study, the self-aggregation process of zinc methyl bacteriopheophorbide(ZMBPhe)-*d* was monitored by a stopped-flow technique. Epimerically pure 3¹*R*- and 3¹*S*-ZMBPhe-*d* self-aggregated in an aqueous tetrahydrofuran (THF) solution as do natural BChls-*c*, *d* and *e* in a chlorosome. We observed a rapid autocatalytic aggregation in a subsecond time scale. Aggregates of the 3¹*R* epimer increased with a change in the Q_y absorption maximum from 698 to 705 nm, suggesting that small aggregates formed as intermediate species. In addition, the rate of aggregation was dependent on the stereochemistry at the 3¹-position of ZMBPhe-*d*; the 3¹*R* epimer self-aggregated more rapidly than the 3¹*S* epimer.

PIII4

A synthetic route to novel porphyrin - cyclam/cyclen conjugates for cancer therapy

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Both porphyrin and cyclam/cyclen molecules are currently used as anti-cancer agents in the areas of photodynamic therapy and radiotherapy respectively. A large research effort is currently focussed on targeted therapy, in which the therapeutic moiety is directed to the tumour, allowing selective killing of cancer cells. A methodology that can be used to link a porphyrin, cyclam/cyclen and biological targeting molecule in one trifunctional unit would be of great interest both synthetically and therapeutically.

Presently, we are developing a synthetic route that will give access to such trifunctional molecules in good overall yield. Presented here are some examples of recently synthesised cyclam/cyclen - porphyrin conjugates incorporating attachment sites for targeting biomolecules, such as single chain fragments of monoclonal antibodies. The synthesis allows a variety of substitution patterns and functionalities to be included on both the porphyrin and macrocycle, allowing optimization of the polarity of the system. Thus, this work opens up the possibility of studying these molecules as trifunctional cancer therapeutics.

PII15**Synthesis and *in vitro* investigation of cationic 5,15-diphenyl porphyrin-mono-clonal antibody conjugates as targeted photodynamic sensitizers**N. Pesa¹, K.A. Smith², H. Savoie¹, J. Greenman², R.W. Boyle¹¹Department of Chemistry & Clinical Biosciences Institute, University of Hull, Kingston-upon-Hull, HU6 7RX, UK;²Postgraduate Medical Institute & Clinical Biosciences Institute, University of Hull, Kingston-upon-Hull, HU6 7RX, UK; email: r.w.boyle@hull.ac.uk

Recognition of photodynamic therapy (PDT) as a promising method for treatment of cancerous tumours and many other diseases has resulted in the development of a number of photosensitizers in recent years. Some of the most important characteristics that have to be considered when designing an ideal PDT-agent are: good phototoxicity with strong absorption at 630-800 nm, lack of significant toxicity in the dark, low skin photosensitivity, high affinity for diseased tissue (while minimal for healthy), and high compound purity with constant composition. We have set out to design and synthesise a range of porphyrins, as new third generation photosensitizers for PDT, that have as many of these characteristics as possible. Our route for targeting specific tissues was *via* bioconjugation to monoclonal antibodies (Mabs) hence we have synthesised a range of photosensitizers that present a single isothiocyanate (NCS) group. The NCS group reacts with lysine residues on the antibody under exceptionally mild conditions; our molecules also contain positively charged moieties to achieve better water solubility, and to induce mitochondrial targeting resulting in induction of apoptosis upon activation with visible light. To improve optical characteristics, chlorin and bacteriochlorin analogues of the cationic isothiocyanato porphyrins are also being prepared.

PII16**Photodynamic inactivation of ion channels formed by mini-gramicidin in bilayer lipid membranes**Y.N. Antonenko¹, E.A. Dutseva¹, E.A. Kotova¹, J.R. Pfeifer², U. Koert²¹Belozersky Institute of Physico-Chemical Biology, Moscow State University, Russia; ²Fachbereich Chemie, Philipps-Universität Marburg, Germany

It has been shown earlier that irradiation of a bilayer lipid membrane (BLM) with visible light in the presence of a photosensitizer leads to reduction of electric current across BLM mediated by gramicidin A (gA). It has been concluded that singlet oxygen generated upon excitation of the photosensitizer damages tryptophan residues of gA, thereby making the peptide incapable to form ion-conducting channels. Here we continued this study with mini-gramicidin, the truncated analogue of gA, in which D-Leu-L-Trp domain of gA is conserved while four amino acid residues from the N-terminus are omitted. Exposure of BLM to a single flash of visible light in the presence of aluminum phthalocyanine provoked a transient decrease in the current across BLM mediated by mini-gramicidin. The time course of this decrease was well fitted by a single exponential with a characteristic time (2.5 s) close to that of gA. According to our previous studies, this parameter corresponds to the single-channel lifetime. Remarkably, the amplitude of mini-gramicidin photoinactivation was 5 times larger than that of gA. In contrast to the covalent dimer of gA showing no current relaxation upon flash excitation, the covalent dimer of mini-gramicidin displayed the flash-induced current decrease, the characteristic time of which shortened as the membrane thickness increased. In the thinnest BLM formed from the DPhPC/squalene solution the time course of photoinactivation was about 10 times longer for the mini-gramicidin covalent dimer than for mini-gramicidin. The photosensitized current relaxation is assumed to reflect equilibration between dimers and monomers and between conducting and non-conducting dimers in the case of mini-gramicidin and mini-gramicidin covalent dimer, respectively.

PII17**Tetraazachlorins - new efficient near infrared photosensitizers for photodynamic therapy**S.V. Barkanova¹, E.A. Lukyanets¹, E.A. Makarova¹, N.B. Morozova², L.V. Umnova¹, R.I. Yakubovskaya²¹Federal State Unitary Enterprise "GNC "NIOPIC", B. Sadovaya 1/4, 123995, Moscow, Russia, phone +07-095-254-95-72, fax +07-095-254-12-00; ²Moscow Hertsen Oncology Institute, 2 Botkinskiy pr. 3, 125284, Moscow, Russia, phone/fax: +07-095-945-87-16

Recently, the interest of the investigators to lipophilic porphyrin-like compounds as active PDT agents significantly increased. Along with porphyrins, chlorins and bacteriochlorins, water-insoluble phthalocyanine derivatives have been tested and their photodynamic efficacy has been demonstrated. Here we report a new class of tetraazachlorins, aza analogues of chlorins, as promising PDT agents for the near IR spectral region. Tetra-methyl-tri-benzotetraazachlorin (H2BTAC, 742 nm/DMF), tetra-methyl-hexaphenyltetraazachlorin (H2TACPh6, 722 nm/DMF), N-methylpyrrolidino[3,4-b]tetraphenyltetraazachlorin (H2PirTACPh, 709 nm/DMF) and N-methylpyrrolidino[3,4-b]tri-(4'-tert-butyl)-benzotetraazachlorin (H2TBtTACPh, 751 nm/DMF) were formulated in Cremophor EL or Proksanol 268 aqueous solutions and tested in mice bearing Erhlich carcinoma or P-388 tumor. All tested compounds exhibit significant photo-induced antitumor activity at doses 0.35-7.0 mg/kg. The tumor growth suppression was not observed without illumination or in the absence of tetraazachlorin.

PII18**Improvement by solubilization in DMPC liposomes of PPME photodynamic effect – A study in human colon cancer cells HCT-116**L. Delanaye¹, C. Volant², N. Jacobs³, R. Greimers³, F. Tfibel⁴, M.-P. Fontaine Aupart⁴, A. Vanderplasschen⁵, M. Hoebeke¹, J. Piette²¹Biomedical Spectroscopy, University of Liège, Liège, Belgium;²Laboratory of Virology and Immunology, CHU of Liège, Liège, Belgium;³Department of pathological anatomy and cytology, CHU of Liège, Liège, Belgium;⁴Laboratory of Molecular Photophysics, University of Paris-Sud, Orsay, France;⁵Department of Immunology and Vaccinology, University of Liège, Liège, Belgium

In view of its potential use in photodynamic therapy (PDT), pyropheophorbide-a methyl ester (PPME) is an attractive second generation photosensitizer derived from chlorophyll a. It has been shown before that liposomes enhance the photodynamic activity of active drugs. This effect is related to the monomerization of most photosensitizers in liposomes because the photosensitizer efficiency was influenced by its aggregation state. We have thus solubilized PPME in aqueous dispersion of small unilamellar DMPC vesicles for our studies in HCT-116 colon cancer cells.

First, we have studied the aggregation process of PPME in ethanol, aqueous solutions and DMPC liposomes using absorption and fluorescence spectroscopy. Our results show that PPME in ethanolic solution was under monomeric form. Analysis of the PPME absorption and fluorescence spectra suggested the existence of aggregates in phosphate buffer (PBS). In DMPC vesicles, fluorescence spectra indicated that incorporation of PPME into the lipid phase induced well dye monomerisation.

According to Matroule and al., necrosis of HCT-116 cells photosensitized to PPME was due to the production of singlet oxygen (¹O₂) and apoptosis was due to the production of other reactive oxygen species (ROS) than ¹O₂. Consequently, a quantitative determination of singlet oxygen and hydroxyl radical production by PPME had been undertaken by electron spin resonance associated with spin trapping technique and absorption spectroscopy. In phosphate buffer, both electron spin resonance and absorption measurements led to the conclusion that singlet oxygen production was not detectable while hydroxyl radical production was very weak. In liposomes and ethanol, singlet oxygen and hydroxyl radical production increased highly; the singlet oxygen

quantum yield was equal to 0.2 in ethanol and 0.13 in liposomes. These results must be related to the triplet-state quantum yield of PPME which was found to be about 0.23 in ethanol, 0.15 in liposomes and too small to be measured in PBS. Our results suggest thus that hydroxyl radical produced apoptosis.

Incubation of HCT-116 cells with PPME in liposomes followed by irradiation with red light increased cellular death as indicated by the Trypan blue exclusion method. Cells in presence of PPME solubilized previously in DMPC liposomes were more fluorescent. FACS analysis of cells stained with Annexin V/PI was used to compare the ratio between apoptosis and necrosis. In presence of liposomes, PPME induced a more important apoptosis than in the case of alone PPME. Moreover, the ratio between apoptosis and necrosis was modified towards apoptosis when PPME was in liposomes. Matroule and al. demonstrated that PPME solubilized in medium culture was mainly localized in the endoplasmic reticulum (ER), the Golgi apparatus (GA) and lysosomes of HCT-116 cells. Thus, the active cellular site for triggering apoptosis mediated by PPME photosensitization was the ER-GA system while mitochondria are the favourite sites for photosensitizers producing apoptosis. Using confocal laser scanning microscopy and organelle specific fluorescent probes, PPME was found to localize in the intracellular membrane system, namely the endoplasmic reticulum, the Golgi apparatus, lysosomes and also in mitochondria.

To conclude, PPME incorporated into liposomes improves the cytotoxic effect of the PDT on HCT-116 cells line by a change in localization, an increase of apoptosis and the increase of hydroxyl radical and singlet oxygen production.

PII19

Photodynamic therapy induces activation and translocation of HIF-1 α as reported by green fluorescent protein *in vitro*

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Photodynamic therapy (PDT) induces the expression of the hypoxia-inducible factor-1 α (HIF-1 α) subunit of the HIF-1 transcription factor and its target gene, VEGF. PDT also induces the expression of the enzyme cyclooxygenase (COX)-2 and its metabolite, prostaglandin (PG). It is known that PGE2 and hypoxia act both independently and synergistically to increase HIF-1 α accumulation and nuclear translocation. It is therefore feasible that PDT-generated oxidative stress may induce HIF-1 α through the PG pathway. To examine the effect of PDT and subsequent PGE2 induction in the activation of HIF-1 α , EMT6 cells were transfected with a plasmid consisting of a hypoxiaresponse- element (HRE) promoter and a downstream green-fluorescent-protein (GFP) gene. HRE is the DNA recognition site for HIF-1. The clones were screened by exposure to cobalt chloride-simulated hypoxia. Monolayers of the selected clone were incubated with 1 μ g/ml Photofrin for 24 h and irradiated with 0.5-2.5 J/cm² 514nm light delivered at 5 mW/cm². Oxygen measurements verified that transient hypoxia was not induced by PDT. 3 h post-PDT, the cells were imaged for GFP expression. We observe a significant increase in GFP intensity above basal levels indicating that PDT activates HIF-1 α . We also observe similarly increased GFP levels in cells exposed to 100 μ M PGE2 for 24 h. These results suggest that under normoxic conditions the PG pathway initiated by PDT-induced oxidative stress may mediate the activation of HIF-1 α . We will verify the relationship between HIF-1 α activation and PG pathway by subjecting cells to PDT in the presence of COX inhibitors. We have also established EMT6 cells transfected with a GFP-tagged HIF-1 α fusion vector to examine the temporal kinetics of the HIF-1 α nuclear translocation in response to PDT. Therefore, using fluorescent reporter genes we explore the role of PDT in regulating HIF-1 α translocation/activation and the underlying molecular pathways. Supported by US NIH grant CA68409.

PII20

Photosensitizer dosimetry reduce inter-subjects variation in photodynamic therapy treatment response

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Effective Photodynamic therapy (PDT) treatments depend on the amount of photosensitizing molecules and the delivered light in the targeting tissue. For the same PDT treatment protocol, variation in photosensitizer uptake between animals may induce variation in the treatment response. This variation can be compensated via control of delivered light dose through photodynamic dose escalations based on online dosimetry of photosensitizer in the animal. Subcutaneous MAT-LyLu Dunning prostate tumor model was used in this study. Photosensitizer BPD-MA uptake was quantified by fluorescence micro-probe at 3 hours after Verteporfin-For-Injection administration. PDT irradiation was carried out after photosensitizer uptake measurement with a total light dose of 75J/cm² and a light dose rate of 50mW/cm². Therapeutic responds of PDT treatments were evaluated by tumor regrowth assay. BPD-MA uptake varied considerably among tumors (inter-tumor variation 56%) and within a tumor (largest intra-tumor variation 64%). Results showed an inverse correlation between the photosensitizer uptake and surviving fraction ($R^2=0.37$). For compensated PDT treatments, photodynamic doses were compensated to lower-quartile, mean and upper-quartile of photodynamic doses in the Non-Compensated PDT treatments by adjusting the total delivered light dose. Variations of surviving fraction decrease from 24.9% in Non-Compensated PDT (NC-PDT) treatments to 16.0%, 14.0% and 15.9% in Compensate-to-Lower-quartile-PDT (CL-PDT), Compensate-to-Mean-PDT (CM-PDT) and compensate-to-upper-quartile-PDT (CU-PDT) treatments respectively. In terms of treatment efficacy, CL-PDT is significantly less effective compared with NC-PDT, CM-PDT and CU-PDT treatments ($p<0.005$). No significant difference in effectiveness was observed between NC-PDT, CM-PDT and CU-PDT. The results indicate that compensated PDT treatments based on photosensitizer dosimetry and dose escalation can reduce the variation in photodynamic treatments response.

PII21

Aggressive tumours might be radiosensitized by porphyrins

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Photodynamic therapy (PDT) began to emerge as a promising alternative therapy for the treatment of early and localized tumors in which adequate local surgery was difficult. But, in order to maximize therapeutic outcome modern cancer treatment usually is a combination of different modalities. In this context combination of PDT with ionizing radiation, especially when photosensitizer can act as radiosensitizer – would significantly enhance the efficiency of PDT.

Thus, the aim of our study was to investigate the possibility to use the same chemical compound – porphyrin – as both a photo- and radio- sensitizer offering unique possibility for a concerted action of the two cancer treatment modalities for a better control of tumor growth.

Two experimental tumor models of different aggressiveness (murine Ehrlich ascite carcinoma and MH-22A hepatoma) were used to investigate the radiosensitization by porphyrins. Data obtained indicate that hematoporphyrin dimethyl ether (HPde), photofrin (PII) and hematoporphyrin derivative (HPD) exert some radiosensitizing properties which are in correlation with purity of

the compound. Of interest to note, that just aggressive EAT was radiosensitized to γ -radiation, whereas no signs of radiosensitization was observed in MH-22A hepatoma tumor. Data obtained support the idea, that dicarboxylic porphyrins, being ligands of peripheral benzodiazepine receptors (responsible for proliferation and highly expressed in aggressive tumors) might induce several sublethal injuries in the cell which further work in concert with ionizing radiation producing additive interaction of two antiproliferative factors.

PII22

PDT-induced changes in angularly resolved light scattering from intact cells as a reporter of mitochondrial and lysosomal morphology

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There has been continuous interest in the details of cell death in the context of photodynamic therapy (PDT). The onset of rapid apoptosis or necrosis in PDT treated cultured cells has been widely observed, and the specific pathway can depend on the treatment parameters and cell line in question. Many photosensitizers localize in, and upon irradiation deposit oxidative stress to, specific organelles such as mitochondria, lysosomes, or endoplasmic reticulum. Damage to these organelle sites is highly significant to the fate of the cell, regardless of which cell death pathway is taken. We present work examining PDT-induced changes in organelle morphology using angularly resolved light scattering from intact cells in suspension. Angularly resolved light scattering is very sensitive to the morphology of the scattering center, and thus has long been used for particle sizing. We demonstrate a Mie theory based interpretation of our measurements from cells that concludes that organelles between 1–4 μm in size are responsible for most of the light scattering signal, and we discuss how organelle-specific PDT insults are used to distinguish the contributions of different organelle populations.

A recent study from our group has demonstrated the ability to detect very early mitochondrial swelling in response to ALA-PDT (Wilson *et al.* Biophys. J. 88 2005), and we have reported seeing similar mitochondrial swelling effects after Pc 4 PDT. Both of these treatment strategies deposit oxidative stress to mitochondria. We will discuss our measurements as a function of total fluence and of time. In addition to scattering changes associated with mitochondria, we have examined the ability to see light scattering changes from the destruction of lysosomes with LS11 PDT. The implications of these measurements in terms of planting these morphology changes in cell death pathways and bulk tissue optical property changes during PDT will be discussed. Supported by US NIH Grant CA68409.

PII23

Time dependent subcellular localisation of mTHPC and apoptotic response in photosensitized MCF-7 cells

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Subcellular localisation of photosensitizers is a main factor involved in apoptotic cell death in response to PDT, since the primary damage sites are closely related to the distribution of sensitizers. In the present study, we have investigated the kinetics of subcellular localisation of mTHPC and apoptotic events in MCF-7 cells, to determine the influence of the time of incubation on the modalities of cell death after photosensitization.

Subcellular localisation, addressed by confocal microscopy, showed that at short incubation times (0.5 h to 3 h) mTHPC displayed diffuse pattern with preferential localisation in the endoplasmic reticulum (ER) and the Golgi apparatus. From 12h it was progressively excluded from the Golgi and at 24 h the

fluorescence topographic profile of mTHPC perfectly overlapped that of the ER probe. Irrespective of incubation time, mTHPC was not prominent in mitochondria and lysosomes.

The apoptotic response to mTHPC-PDT (650 nm), addressed by flow cytometry, suggested that apoptosis measured 24 h after PDT at LD₉₇, decreased from 40 % at 3 h to 20 % at 24 h. The kinetic of mitochondria events at different times post-PDT, performed by flow cytometry, were different according to the time of incubation. The concomitant profiles of loss of mitochondrial membrane potential ($\Delta\psi\text{m}$) and release of cytochrome C were perfectly correlated with that of photocytotoxicity at 24 h and 48 h post-PDT, for 3 h and 24 h incubation, respectively. These data suggest that it is possible to modulate the mechanism of cell death, which may be relevant in the clinical context.

PII24

Caspase-2: a possible trigger of apoptosis induced by ZnPc photodynamic treatment in A-549 cells

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Photodynamic treatments therapy applied to cell cultures represent a widely accepted experimental method to investigate molecular mechanisms that lead to apoptotic cell death. To this respect, the subcellular localization of photosensitizers seems to be a significant factor in order to determine the apoptotic pathway that could be activated.

We have characterized the experimental conditions that lead to apoptotic cell death in A-549 cells incubated with ZnPc and irradiated with red light. Previously we have found that in this cell line the drug is localized in the Golgi apparatus after 3 h incubation. Indirect immunofluorescence analysis of the events that lead to apoptosis, reveal caspase-2 activation in the Golgi region immediately after photodynamic treatments. Few minutes later, this organelle started to disrupt and 6 h after treatment the nuclei resulted affected, showing the fragmented morphology typical of apoptosis. From these results we could deduce that caspase-2 activation could initiate the cell death process and, like other cell organelles, the Golgi apparatus could have its own sensors for cellular stress, being involved in a signaling balance that would determine either cell death or cell survival.

PII25

Time-resolved singlet oxygen phosphorescence detection in cells using kHz diode-pumped solid-state lasers

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Time-resolved detection of singlet oxygen in biological systems is a long sought-after goal given the participation of this reactive oxygen species in many biological processes, e.g. photodynamic therapy. The ability to monitor the dynamics of singlet oxygen in biological systems would help elucidate its role in such processes, thereby providing the basis for a more rational design of new photosensitizing drugs for photodynamic therapy.

In recent years, two major technical advances have appeared in the field of singlet oxygen detection: the first one is the commercial availability of near-IR photomultiplier tubes that allow photon counting detection of singlet oxygen phosphorescence at 1270 nm. They provide a substantial increase in sensitivity compared to germanium or indium gallium arsenide photodiodes. In addition they are substantially faster (ca. 2 ns time-response) allowing the resolution of submicrosecond events.

The second technical advance is the development of inexpensive pulsed diode-pumped solid-state lasers working in the KHz regime. They allow the accumulation of millions of signals in relatively short times, thereby further increasing the sensitivity of the detection setup.

The combination of these two innovations has allowed us to monitor the dynamics of singlet oxygen produced in the membrane of human skin fibroblasts.

Acknowledgements: this work has been supported by the Spanish Ministerio de Ciencia y Tecnología (SAF 2002-04034-C02-02).

PII26

Binding of cationic porphyrin to double stranded viral DNA analyzed by comprehensive spectroscopic methods

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Three main application fields of the cationic porphyrins have been developed in the last decade. These are the DNA-targeted photodynamic inactivation of microorganisms, the probing the secondary and tertiary structure of nucleic acids, and recognition of biomacromolecules using nanoparticles functionalized with cationic side chains. For these tasks, detailed characterization of the environmental factors influencing the magnitude and the mode of the DNA-binding is of particular importance.

We investigated the complexation of tetrakis(4-N-methylpyridyl)porphyrin (TMPyP) with free and encapsidated DNA of T7 bacteriophage. To identify binding modes and relative concentrations of bound TMPyP forms, the porphyrin absorption spectra at various base pair/porphyrin ratios were analyzed. Spectral decomposition, fluorescent lifetime, and circular dichroism measurements proved the presence of two main binding types of TMPyP, e. g., external binding and intercalation both in free and in encapsidated DNA. TMPyP binding does not influence the protein structure and/or the protein – DNA interaction. Concentrations of TMPyP species were determined by comprehensive spectroscopic methods. Our results facilitate a qualitative analysis of TMPyP binding process at various experimental conditions. We analyzed the effect of base pair composition of DNA, the presence of protein capsid and the composition of buffer solution on the binding process.

PII27

An ESR study on type I and type II photoreaction induced by neutral and cationic porphyrin derivatives

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Photodynamic treatment (PDT) is a combination of light and light sensitive molecules such as porphyrins. Upon irradiation of photosensitizers, reactive oxygen species (ROS) are generated in the PDT leading to anticancer and antiviral activities. The light activated sensitizer can undergo two kind of reaction. It can react directly with neighbouring molecules and transfers an electron to produce radicals (type I reaction) or the triplet can transfer its energy directly to oxygen to form singlet oxygen (type II reaction). The ability of 5,10,15-(4-β-D-galactosylphenyl),20-(2',3',4',5'-pentafluorophenyl)porphyrin (TPFP) and meso-tetrakis(4-N-methylpyridyl)porphyrin (TMPyP) to generate ROS by type I. and/or type II. mechanism was investigated in phosphate buffer. After visible irradiation of porphyrin solution under aerobic conditions at pH 7.4, the formation of singlet oxygen (¹O₂) and superoxide anion (O₂^{•-}) radicals was concluded using electron spin resonance (ESR) with the spin trap 5,5-dimethyl-1-pyrroline-1-oxide (DMPO). The combination of visible light and photosensitizer in the presence of spin trap DMPO gave the characteristic ESR spectrum of DMPO-hydroxyl radical spin adduct DMPO-OH. No signal was observed in the dark and under anaerobic conditions (bubbling with Ar). After addition of ethanol (2%) the DMPO-OH signal intensity disappeared and the ESR spectrum corresponding to CH₂CHOH• radical was detected. Using ¹O₂ quenching compound (sodium azide) and O₂^{•-} quencher

(superoxide dismutase) we tested whether singlet oxygen or superoxide anion is involved in the photoreaction of the porphyrin derivatives.

PII28

Systemic suppression of contact hypersensitivity in mice induced by products of merocyanine 540 photolysis

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Photosensitizer merocyanine 540 (MC540) is used in photodynamic therapy to treat some forms of cancer. It is known that the need for simultaneous exposure of tumors to MC540 and light could be circumvented by prior exposure of the solution of MC540 to light and subsequent use of photoproducts of MC540 (pMC540) as anticancer drug [Gulliya et al., Eur. J. Cancer 1990, 26, 551-553]. It was shown that pMC540 induced apoptosis of tumor cells [Pervaiz et al., Cancer Lett. 1998, 128, 11-22] which was considered as the main mechanism of pMC540 antitumor activity. However, there were no reports concerning effects of pMC540 on immune system. We found that pMC540 modulated T cell immunity. We demonstrated immunomodulative activity of pMC540 by employing the models of Contact Hypersensitivity (CHS) to dinitrofluorobenzene (DNFB) or oxazolone and Delayed Type Hypersensitivity (DTH) to sheep erythrocytes in mice. pMC540 strongly modulated the DTH response, i.e. the DTH was enhanced at low doses and inhibited at higher doses of MC540 preirradiation. The CHS response was inhibited by intravenous injection of pMC540 in fluence dependent manner: the higher was the fluence the higher was suppression. Not irradiated MC540 did not influence the DTH or the CHS. To evaluate immune mechanisms of CHS suppression by photoproducts of MC540 we performed experiments on adoptive transfer of effector cells as well as on transfer of suppressive effect itself. It was found that photoproducts of MC540 *in vivo* induced: a) antigen non-specific suppression of the CHS; b) suppression of functions of CHS effector cells; c) activation of cells with suppressive potential. Thus effects of pMC540 on T cell immunity should be taken into account when this drug is used for the treatment of cancer.

PII29

Photothermal therapy of experimental tumours using Pd(II)- and Pt(II)-octabutoxy-naphthalocyanines as sensitizers

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In the frame of our ongoing investigations on the molecular factors which control the efficiency of photothermal sensitisation, we identified Pd(II)- and Pt(II)-octabutoxy-naphthalocyanines (PdNc, PtNc) as efficient photosensitising agents. Actually, the two Ncs were found to be accumulated in significant amounts by B78H1 cells: e.g., 48 h incubation of the amelanotic melanoma cells with 5.1 μM PdNc or PtNc yielded a cell uptake of 1.46 and, respectively, 0.98 nmoles of naphthalocyanine per mg of cell protein. Subsequent 20 min.- irradiation (Ti:sapphire laser, 826 nm and 809 nm, respectively, for PdNc and PtNc; 30 ns pulses, 10 Hz; 120 mJ/pulse) of the Nc-loaded cells caused a 84.5% (PdNc) and 69% (PtNc) drop in cell survival. Repeating the irradiation experiments under identical conditions for 18 h-incubated cells which had a very similar endocellular concentration of both Ncs showed a less extensive cellular inactivation (48% for PdNc, and 23.9% for PtNc) in agreement with previous data showing that the incubation time modulates both the pattern of naphthalocyanine subcellular distribution and its photothermal sensitisation efficiency. In all cases, SEM analysis of photosensitised cells showed that the photoprocess leads to the fast ejection of a large amount of cytoplasmic material, in agreement with our previous findings on the mechanism of photothermal sensitisation typical of

NiNc. Hence, the generation of acoustic shock waves appears to be a generally valid mechanism for photothermal sensitisation. PdNc proved to be also an efficient photothermal sensitizer *in vivo*. Thus, i.v.-injection of 1.8 mg/kg body weight of liposome-incorporated PdNc to C57 mice bearing a subcutaneously transplanted pigmented melanoma followed by 826 nm-light irradiation in a pulsed regime (30 ns pulses, 10 Hz, 100 mJ/pulse) caused an important tumour response; thus, at 20 days after photothermal treatment all six mice appeared to be tumour-free, and four out of six mice were still tumour-free after 30 days.

PII30

Antitumour properties of irradiated visible light active titanium dioxide photocatalysts

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Photocatalytic properties of titanium dioxide have been studied over the last 25 years. Recent investigation showed successful applications of titanium dioxide in removal of organic and inorganic contaminants of water and air. Titania may be applied as component of self-cleaning and self-sterilising surfacesⁱ.

Nowadays an increasing interest in TiO₂ applications for neoplastic and bacterial cells killing is observed. In our studies the photodynamic properties of irradiated titanium dioxide were examinedⁱⁱ. Photogeneration of reactive oxygen species (ROS) from photoexcited semiconductor may lead to a cellular death (apoptosis or necrosis). Free radicals like hydroxyl radical, superoxide and others may initiate autooxidation of lipids, cause other cell components destruction and DNA strand breakage leading to cell deathⁱⁱⁱ.

In the present study we have focused on phototoxic properties of titanium dioxide modified with selected coordination compounds on melanoma (S91) and mouse macrophages (RAW 264.7) cell lines. Titanium dioxide was modified at the surface with selected coordination compounds. The photokilling properties of these materials showing photocatalytic effect of organic pollutants mineralization under visible light irradiation^{iv}, were tested *in vitro* as well as in the micelles 2% Triton X-100 model system.

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PII31

Nitric oxide-induced long-term protection of tumor cells against photooxidative killing

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We showed recently that photokilling of COH-BR1 tumor cells that had been metabolically sensitized with protoporphyrin IX (PpIX) is strongly inhibited by spermine NONOate (SPNO)-derived NO delivered concurrently with light exposure (Photochem. Photobiol. 78: 262, 2003). This effect was correlated with NO's ability to suppress photoinduced chain peroxidation of cell membrane lipids. Here we report that NO also elicits a delayed response with COH-BR1 cells lethally challenged with t-butyl hydroperoxide or PpIX-sensitized photodynamic action. Twenty hours after exposure to 0.2 mM SPNO, cells were found to be substantially more resistant than

non-treated or decomposed SPNO-treated controls. Resistance developed gradually after NO was introduced, none being seen at 4 h, ~50% at 10 h, and 100% at 18-20 h. Intracellular free iron level determined with the fluorescent probe calcein was significantly elevated ~3 h and then reduced 24 h after SPNO addition, accompanied by induction of heme oxygenase-1 (HO-1) and ferritin. Western blot analysis showed an early increase in HO-1 level (2 h after NO exposure), followed by gradual accumulation of ferritin, reaching a maximum 20 h later. The protein upregulation was consistent with a cytoprotective mechanism involving mobilization of "signaling" iron. Importantly, NO generated by activated RAW 264.7 macrophages on porous inserts also induced a long-term photoresistance in underlying COH-BR1 cells. The delayed protective effects of NO on photodynamically-challenged cells could impact negatively on the antitumor efficacy of photodynamic therapy mediated by PpIX and other sensitizers. (Supported by NIH Grant CA70823 and MNI Grant 2P04A04428)

PII32

Apoptosis accommodating effects of nitric oxide (NO) in photodynamically stressed tumor cells

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Using COH-BR1 tumor cells, we have developed two different sensitization protocols for 5-aminolevulinic acid-derived protoporphyrin IX (PpIX), one (Pr-1) in which PpIX is still mainly in mitochondria (Mito) and the other (Pr-2) in which much of it is allowed to diffuse to other target sites, including plasma membrane (PM). Using the nuclear fluorophores Hoechst and Propidium Iodide as indicators, we found that cells prepared by Pr-1 died mainly by apoptosis after irradiating with broad-band visible light, whereas those prepared by Pr-2 died mainly by necrosis. Introduced at a non-toxic concentration (0.2 mM) immediately before light, the NO donor spermine NONOate (SPNO) inhibited photoinduced necrosis of Pr-2 cells by ~75%. Interestingly, most of the remaining cell death (>70%) was apoptotic rather than necrotic, as assessed by caspase-3 activation and chromatin condensation. We deduce from these and related findings that NO, acting as a chain-breaking antioxidant at the PM, and thus inhibiting permeabilization, helped to preserve a level of energy metabolism that was necessary to accommodate Mito- or PM-initiated apoptosis. (Supported by NIH Grant CA70823 and MNI Grant 2P04A04428)

PII33

Influence of aggregation, pH and environment on photostability of TPPS₄: spectroscopic study

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Meso-tetra(4-sulphonatophenyl)porphine (TPPS₄) is a well known 2-nd generation sensitizer used in the photosensitized therapy. Optical properties of TPPS₄ in different medium significantly depend on concentration and pH. TPPS₄ localizes in lysosomes where pH is around 4. The ionic equilibrium constant (pK_a) of TPPS₄ is 4.8, therefore inside the cells the sensitizer could exist in protonated form (TPPS₄²⁺), which possesses specific aggregation properties. It is important to determine how variation in medium acidity, aggregation state and microenvironment affects TPPS₄ photostability.

The studies were performed in phosphate buffered solutions (pH=7,0), in aqueous solutions of different acidity, and in model biological media containing bovine serum albumin (BSA). Absorption spectroscopy methods were applied. Samples of TPPS₄

(5×10^{-6} – 5×10^{-5} M) were irradiated with light sources (diode lasers $\lambda_{em} = 635, 652$ nm, Ar⁺ laser $\lambda_{em} = 488$ nm and LED $\lambda_{em} = 492$ nm) at fluence rate 10–100 mW/cm² in 1 cm cuvettes. It was found that TPPS₄ is more photostable in acidic than in neutral solution (pH 4–7). The same effect was observed in the presence of BSA, however the photobleaching of TPPS₄ absorbance was generally enhanced as compared to pure solutions. Moreover, the presence of BSA induced photobleaching of TPPS₄ in acid medium, where it is completely photostable otherwise. The photostability sequence for different species was TPPS₄²⁺ > TPPS₄²⁺+BSA > TPPS₄ > TPPS₄+BSA. The decrease of oxygen concentration during irradiation revealed that photodestruction was caused by type II photochemical reactions.

In highly acid media TPPS₄²⁺ tends to form J-aggregates self-assembled into tubular nanostructures. Irradiation induced small absorbance changes of J-aggregates at high porphyrin concentration, while in less concentrated solutions relatively fast absorbance bleaching of J-aggregates and a plateau phase were observed. We presume that the irradiation destroys nanostructures of J-aggregates.

PII34

Application of N-acetyl-3, 7-dihydroxyphenoxazine as singlet oxygen and hydrogen peroxide sensor in photodynamic reactions

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The N-acetyl-3,7-dihydroxyphenoxazine (A-Phe), produced by Molecular Probes under commercial name Amplex Red™ is widely applied in biochemical investigations for hydrogen peroxide detection. In the presence of peroxidase acting as a catalyst it reacts with hydrogen peroxide to form highly fluorescent dye – resorufin. In our work we applied A-Phe and sulphonated aluminum phthalocyanine (Photosense™) as photosensitizer produced by NIOPIK to study singlet oxygen and hydrogen peroxide formation in photodynamic reactions. We have found out that A-Phe is transformed to resorufin even in the absence peroxidase due to singlet oxygen mediated reactions during light irradiation with Photosense. To our knowledge it is the first experimental observation of dye transformation from nonfluorescent to fluorescence form affected by singlet oxygen. Further, in course of light irradiation the fluorescent form (resorufin) is photobleached by singlet oxygen, forming other nonfluorescent substance. The fluorescence dynamic has fast outburning at initial stage after starting light irradiation, pass through pronounced maximum and then goes down due to photobleaching. Moreover, the fluorescence outburning and photobleaching dynamic are more distinct in D₂O than in H₂O suggesting the singlet oxygen involvement.

We also have found out that in the presence of peroxidase the fluorescence outburning is increased suggesting the formation of hydrogen peroxide in photodynamic reactions. The involvement of hydrogen peroxide in photochemical reactions may be important for the damage mechanism during photodynamic therapy. Wentworth et al. observed the transformation of singlet oxygen into hydrogen peroxide in water catalyzed by antibodies and claimed that this mechanism may be essential in immune defense reactions. We also studied the formation of hydrogen peroxide during light irradiation of plasma proteins in the presence of Photosense as photosensitizer. The results obtained are discussed.

PII35

Heme oxygenase-1 protects tumor cells against PDT-induced toxicity

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Photodynamic therapy (PDT), a promising therapeutic modality for the management of solid tumors, is a two-phase treatment consisting of a photosensitizer, oxygen and a visible light. Increasing evidence indicates that tumor cells in regions exposed to sublethal doses of PDT can respond by rescue responses that lead to insufficient cell death. Using a cDNA microarray analysis we identified gene products overexpressed in tumor cells exposed to PDT. One of these was heme oxygenase-1 (HO-1), a rate limiting enzyme involved in the degradation of heme into carbon monoxide, free iron and biliverdin. We decided to examine the role of HO-1 overexpression in the effectiveness of PDT and to investigate whether inhibition of HO-1 affects the antitumor activity of this treatment regimen. In the initial experiments we observed overexpression of HO-1 protein in cells exposed to PDT. Chemical induction of HO-1 with hemin resulted in a decreased sensitivity of the tumor cells to the cytotoxic effects of PDT. Inhibition of HO-1 activity using Zn(II) protoporphyrin IX – a competitive HO-1 inhibitor, led to potentiated antitumor effectiveness of PDT. We also established a tumor cell line stably transfected with HO-1 gene. These cells were more resistant to PDT than controls in *in vitro* as well as *in vivo* models. Leukocyte infiltration after PDT in the tumor sites composed of HO-1 transfectants was significantly delayed. The protective role of HO-1 in PDT-treated tumor cells is not dependent on the increased production of the antioxidant biliverdin or bilirubin or generation of carbon monoxide. The mechanism of this interaction probably relays on the production of free iron and ferritin heavy chains that exert cytoprotection. We conclude that inhibition of HO-1 activity is an effective treatment modality capable of potentiating the antitumor effectiveness of PDT.

PII36

On the role of calcium elevation in glioblastoma cell death under hypericin-induced photodynamic treatment

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Photodynamic therapy (PDT) involves a photosensitizing drug that, upon light irradiation and presence of oxygen, results in tissue damage such as tumor destruction. PDT has been investigated as an effective treatment in the neuro-oncological field. Most forms of brain tumor have a glial origin therefore it is very important to study the mechanisms of PDT-induced death of glioma cells. Cell death caused by PDT can occur either by apoptosis or necrosis depending on the cell type, concentration, intracellular localization of the sensitizer and the light dose and managed by several signaling pathways. Photodynamic therapy of various cell types has been shown to raise the [Ca²⁺]_i which is involved in different signaling processes and may eventually lead to cell death. Recently

hypericin, polycyclic quinone, has been proposed as a photosensitizer for treatment of some kind of tumors.

In the present work we studied the involvement of calcium elevation in the glioblastoma cell death under hypericin induced photosensitization. Cells were co-incubated with the photosensitizer and the calcium-sensitive probe Fluo-3 for measuring calcium changes or with Mito-tracker Green for colocalization of hypericin. Colocalisation studies have been shown that hypericin was localized predominantly in the mitochondrial membranes. Confocal laser scanning microscopy revealed two transient increases of the cytoplasmic calcium concentration: The first increase occurred 0.5 min and the second increase occurred 1.5 min following PDT irradiation. The intracellular calcium concentration increase was accompanied by blebbing, subsequent shrinking and cell death. It is known that mechanism of PDT-action strictly depends on the localization of the photosensitizer. From this the intracellular calcium increase could be due to photodamage of mitochondria in the case of hypericin. On the other hand mitochondrial injuries as well as intracellular calcium elevation are main mechanisms which initiate apoptotic processes. These findings suggest that an increase of intracellular calcium concentration due to light induced injury of mitochondria might be a key event in the death mechanism of photosensitized glioblastoma cells.

The work was supported by European Society for Photobiology.

PII37

Singlet oxygen generation by selected phthalocyanines and naphthalocyanines

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In the last decade there has been an increase in research on the so called second-generation photosensitizers, which exhibit improved spectroscopic, photobiological and pharmacokinetic properties as compared with Photofrin and other porphyrins.

Phthalocyanines and Naphthalocyanines are considered as part of this group as a consequence of their intense absorbance in the clinically useful red spectral region, high photosensitizing activity, and good selectivity of tumour targeting. They are also well known as efficient type II photosensitizers, however, some photophysical properties such as steady-state absorption, fluorescence and singlet oxygen quantum yields can be affected by the hydrophobicity of the compounds and also by the nature of the central metal ion.

The hydrophobicity effect was studied on five different Si (IV)-naphthalocyanines with hydrophobicity modulated through five different axial ligands (from six long alkyl chains to four short alkyl chains and two amino groups, and four short alkyl chains and two maltose residues).

The effect of central metal ion was studied on (O-Bu)₈ substituted naphthalocyanines (Nc) and Phthalocyanines (Pc): Zn(II)Nc(O-Bu)₈; H₂Nc(O-Bu)₈; Ni (II) Pc(O-Bu)₈ and on Zn(II)Pc-(O-Dec)₈. The singlet oxygen quantum yields was determined by steady state photolysis of dimethylantracene (DMA) sensitized by the different compounds. The singlet oxygen quantum yield of Zn(II)Pc was used as reference.

Acknowledgments: this work received financial support from Fondecyt (Chile), Grants 1040667 and 7040136.

PII38

Fluorescence spectroscopic study of hypericin-photosensitized oxidation of low-density lipoproteins

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Low-density lipoproteins (LDL) play a key role in the delivery of hydrophobic photosensitizers to tumor cells in photodynamic therapy (PDT). Hypericin (Hyp) is a natural photosensitizing pigment which displays an antiproliferative and cytotoxic effect on tumor cells as well as virucidal activity against several types of viruses. Thus, the study of interaction of Hyp with LDL could provide useful information about mechanism and physiological relevance of Hyp application in PDT and photodiagnosis. By means of spectroscopic study we demonstrate that Hyp interacts nonspecifically with LDL, most probably with lipid fraction of LDL. The molar ratio of monomeric Hyp binding to non-oxidized LDL and oxidized LDL is 30:1. Further increasing of Hyp concentration leads to the formation of Hyp aggregates inside LDL molecule. We do also demonstrate that photoactivated Hyp oxidize LDL in a light dose and excitation wavelength dependent manner and that the level of oxidation of LDL depends on the amount of monomeric Hyp inside LDL molecule. The maximum of the photosensitized oxidation of LDL by Hyp is achieved for a 30:1 molar ratio, which corresponds to the maximum concentration of monomeric form of Hyp in LDL.

Acknowledgment: this work was supported by the Slovak Science and Technology Assistance Agency, grant No. APVT-20-036104 and by the Scientific Grant Agency of the Ministry of Education of Slovak Republic grant No. 1/2278/05.

PII39

Photothermal sensitisation of mammalian cells with nickel-octabutoxy-naphthalocyanine

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Photothermal sensitisation is a novel approach to photodynamic therapy of cancer. It takes advantage of the property of certain photosensitisers (either endogenous or exogenous) to transform light energy into heat, which spreads as an acoustic shockwave. The heat production during the short decay time of the excited photosensitiser produces temperature increases of 100-150°C. This requires the use of pulsed laser light with high fluency rates. Long or repetitive treatments demand photostability of the thermal photosensitiser. Nickel (II)-octabutoxy-naphthalocyanine (NiNc) is a synthetic chromophore with a high absorbance peak at 850 nm ($\epsilon=280000 \text{ M}^{-1}\text{cm}^{-1}$). It is rather photostable and its adequacy as a potential cell inactivation agent was tested on diverse human and murine cell lines. The use of 7.7 μM NiNc concentration and long incubation times (18h and 48h) causes massive cell death. It seems to occur on all the cells that incorporate the NiNc liposomes into aggregated vesicles in a precise cellular district. Cell death appears to be due to a cell disruption mechanism. The disruption is caused by the ejection of large cytoplasmic mass containing the aggregated NiNc previously incorporated by the cell. This ejection is observed in scanning electron microscopy images, but also in bright field microscopy. In this last case it is possible to correlate the presence of the photosensitiser with the ejection areas.

PII40

Mechanisms of uptake of a water-soluble anionic zinc phthalocyanine in murine fibrosarcoma cells (RIF-1)

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Phthalocyanines are promising second generation photosensitisers for use in photodynamic therapy. The biological effects of these molecules can be altered following modification of their peripheral groups and a large number of derivatives have been developed. The water soluble phthalocyanines are of particular interest because of the ease of formulation and administration. There are very few studies of the detailed mechanisms of photosensitiser uptake apart from those sensitizers that are known to bind to the serum lipoproteins. The mechanism of uptake of these sensitizers is thought to involve LDL- receptor mediated uptake to varying degrees. The mechanism of uptake of water soluble sensitizers has not been studied in any detail.

We have studied the uptake of a water soluble zinc phthalocyanine in a mouse fibrosarcoma cell line. In particular we have investigated the participation of diffusion and endocytic processes dependent and independent of clathrin.

The uptake of a β -alanine derivative of zinc phthalocyanine by RIF-1 cells was studied under various specific conditions and was quantified fluorometrically and visualized by fluorescence microscopy.

Cellular accumulation was found occur by both a clathrin dependent and a clathrin independent endocytic uptake. Little evidence for diffusion, caveolae-mediated, macropinocytic or phagocytic uptake was seen under the conditions investigated. Involvement of a receptor to mediate phthalocyanine uptake is unlikely to exist because of the high apparent K_m value (268 μ M), thus the uptake appears to be via a non-specific endocytic route. As uptake is concentration dependent this mechanism could be adsorptive endocytosis, preceded by non-specific interaction of the photosensitiser with the cell membrane.

Understanding the mechanism of photosensitiser uptake may enable future drugs to be developed with improved uptake.

PII41

m-THPBC potential as photosensitizer for liver PDT

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The aim of this study was to evaluate the *in vivo* possibility to use *m*-THPBC as a treatment for liver PDT. Only few *in vivo* properties of this molecule are known such as photodynamic activity and the necrosis volume of *m*-THPBC-PDT. Therefore we studied the light penetration in the liver and biodistribution, pharmacokinetics and photobleaching of *m*-THPBC. The animal model was BALB/c nude mice bearing WiDr human adenocarcinoma xenografts after i.v. administration of *m*-THPBC at 0.5 mg kg⁻¹. Light penetration in the liver was measured from 500 nm to 800 nm. Penetration of light at the *m*-THPBC excitation wavelength (740 nm) was shown to be 30% higher than the light penetration at the *m*-THPC excitation wavelength (650 nm). *m*-THPBC and *m*-THPC photobleaching was measured in the skin of the mouse 24 h after injection by the mean of light induced fluorescence spectroscopy (LIFS). Irrespective of the sensitizers, photobleaching kinetics rather fitted a bi-exponential decay, suggesting that the two photosensitizers are both under monomeric and an aggregated form. The photobleaching rates are 4 times higher for *m*-THPBC than *m*-THPC.

Biodistribution experiments measured with LIFS, demonstrate a good accumulation in the tumor and a higher uptake of *m*-THPBC for the liver than *m*-THPC. Photobleaching rates and biodistribution characteristics of *m*-THPBC are very promising in terms of therapeutic ratio, since illumination during PDT could reduce its concentration in the normal tissues to a level below the

photodynamic threshold, while sufficient sensitiser could remain in the tumour to sensitise its photodestruction.

m-THPBC skin pharmacokinetic reveals an identical kinetic as this of *m*-THPC with a maximum at 72 hours. This result together with the higher photobleaching rates of *m*-THPBC compared to *m*-THPC are very interesting in terms of skin photosensitivity.

Taken as a whole this results offers promising therapeutic perspectives for *m*-THPBC-PDT for liver tumors.

PII42

Development of sensitizers based on squaraine moiety for photodynamic therapy

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Photodynamic therapy (PDT) is a non-invasive technique for the treatment of both neoplastic and non-neoplastic diseases by the combined action of light and a photosensitizing drugⁱ. The photosensitizer, when injected into the body, due to its inherent properties accumulates in the cancerous tissues and on irradiation with light of suitable wavelength generates cytotoxic agents, which cause tumor necrosis. Contrary to the conventional treatments such as chemotherapy and radiotherapy, PDT is relatively a safer treatment since the induction of tumor necrosis ceases when the light is switched off. The recent advent of laser fiber optics, endoscopy and laparoscopy has made it possible by PDT to alter only the irradiated area with minimal systemic toxicity, thereby extending the clinical application of PDT to a variety of cancers. Several photosensitizers including porphyrins, metallophthalocyanines, chlorins, porphycenes, purpurins and aminolevulinic acid-mediated porphyrins have been extensively studied for their use in PDT. With the 1st generation photosensitizer, Photofrin[®] already in clinical use and several other photosensitizers under various phases of clinical trials, the search for more effective photosensitizers has become an important area of research in recent years. In this context, we have designed and synthesized a few heavy atom substituted squaraine dyes and have investigated their photophysical and photobiological properties under different conditions. These dyes possess favorable photophysical properties and generate cytotoxic agents such as singlet oxygen in quantitative yields^{ii-iv}. Cytotoxicity and mutagenicity studies in mammalian cells and bacterial strains revealed that these dyes are non-toxic in the dark but exhibit high cytotoxicity only when activated with visible light^{v-vi}. Cytotoxicity and DNA damage studies in cellular and cell-free conditions revealed that singlet oxygen is the major reactive species responsible for the photobiological activity of these dyes^{vii}. These results clearly indicate that sensitizers based on squaraine moiety could form an effective alternate system to the well-studied porphyrin based sensitizers for potential PDT applications.

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PII43

QSAR modeling and prediction of tumoricidal activity of aryl-porphyrins in photodynamic therapyE. Papa¹, P. Gramatica¹, S. Banfi², E. Caruso²University of Insubria, DBSF- Varese, Italy; ¹QSAR Res. Unit,http://www.qsar.it; ²Organic Chemistry Unit

The *in vitro* activities on human colon adenocarcinoma cells line (HCT116) of a series of 34 mono-, di- and tetraaryl-porphyrins used as photosensitizers in photodynamic therapy have been modelled and predicted on the basis of their molecular structure, using multiple linear regression (MLR).

Theoretical molecular descriptors were calculated from the three-dimensional structure (3D) of the geometry-optimized molecules. The selection of the descriptors more correlated to the studied biological activity has been obtained by applying the Genetic Algorithm approach.

Various QSAR (Quantitative Structure-Activity Relationships) models, based on different combinations of molecular descriptors and with comparable satisfactory modelling quality have been obtained: among them the best QSAR model has been selected by applying different kind of statistical validation. The models have been validated both internally by leave-one-out (LOO) cross-validation, by bootstrap and by Y-randomization, both externally by splitting the available set of 34 experimental data in 23 training chemicals for model development and 11 validation chemicals for model evaluation. The splitting in representative and structurally balanced sets has been performed by Kohonen Map-Artificial Neural Network (K-ANN). Only the QSAR model verified for its high predictive performance on an external set of chemicals is here proposed as applicable for prediction purposes.

The model is based on 3 molecular descriptors of different structural features (2D and 3D) and has good performances: $R^2=0.841$, $Q^2=0.788$, $Q^2_{\text{boot}}=0.778$, $Q^2_{\text{ext}}=0.789$. On the contrary log Kow is very poorly correlated to the response. The model, applied to 4 not yet synthesized chemicals, predicts an high activity for 3 monoaryl-porphyrins and a low activity for one tetraaryl-substituted. The application of the proposed QSAR model, validated for its predictivity, can be useful for the synthesis design of new promising photosensitizers.

PII44

Halogenated water-soluble porphyrins and their photodynamic action in melanoma cellsL.G. Arnaut¹, J.M. Dabrowski², C. Monteiro¹, A. Peixoto¹, M.M. Pereira¹, S.J. Formosinho¹, G. Stochel², K. Urbanska²¹Departamento de Química, Universidade de Coimbra, 3049 Coimbra, Portugal; ²Faculty of Chemistry, Jagiellonian University, Krakow, Poland

Halogenated porphyrins exhibit long triplet lifetimes and increased singlet oxygen quantum yieldsⁱ. The same properties were also observed for halogenated chlorinsⁱⁱ and bacteriochlorinsⁱⁱⁱ. Although such properties are desirable for PDT, they must be combined with a controlled solubility in aqueous media. In this work, we report the photophysical properties of new water-soluble halogenated porphyrins, namely *meso*-tetrakis(2-chloro-3-sulfophenyl)porphyrin (TCPPSO₃H) and *meso*-tetrakis(2,6-dichloro-3-sulfophenyl)porphyrin (TDCPPSO₃H)^{iv}, such as triplet lifetimes and fluorescence and oxygen singlet quantum yields. In addition, we report *in vitro* essays in S91 (melanoma, mouse), SKMEL 188 (skin, melanoma, human) and MCF7 (breast, adenocarcinoma, human) cell lines that demonstrate the low toxicity of TCPPSO₃H in the dark and its remarkable photodynamic effect^v.

These preliminary results demonstrate that TSPPO₃H is a potent PDT agent. Further *in vivo* and clinical studies will be necessary to materialize its potential.

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PII45

Combined action of Visudyne and coherent or non-coherent light on melanoma cellsP. Nowak-Sliwinska^{1,2}, G. Stochel¹, K. Urbanska²¹Faculty of Chemistry; ²Faculty of Biotechnology, Jagiellonian University, Cracow, Poland

The potential application of photodynamic therapy (PDT) in the treatment of malignant melanoma is still under investigation. This therapy has a high probability of success and offers clear advantages over existing treatments because it is a non-invasive procedure that can treat multiple tumours simultaneously. The main directions in further studies are concentrated on a search of an efficient photosensitizer and appropriate light source. Since melanoma grows in the skin or in the choroid, the deep-penetrating coherent light is not mandatory. The application of non-coherent light source can be advantageous since an illumination field is wider than for laser systems.

The aim of this investigation was to compare the efficiency of photodynamic effect (PDE) in pigmented melanoma cells (S91/13 subline) after combined action of Visudyne (Verteporfin) as a photosensitizer and coherent or non-coherent light.

To check the cytotoxicity of Visudyne, melanoma cells were incubated in media containing different concentrations of the dye. To establish the PDE the cell cultures were incubated in the sublethal dye concentration and irradiated by laser (689 nm, 20 J/cm², 100 mW/cm²) or a halogen lamp (43.2 J/cm², 72 mW/cm²). 48 hours later the cell number was determined. For both light sources the results were comparable. The surviving fraction of the irradiated cells was about 10-fold lower in comparison with non-irradiated, treated with the same concentration of the compound.

The current study has shown the significant PDT effect on pigmented melanoma *in vitro* after combined action of Visudyne and coherent or non-coherent light and set the basis for future studies in the tumour model.

PII46

Complete model of oxygen transport in photodynamic therapy: a simulation of oxygen dynamics *in vivo*K.K.-H. Wang¹, S. Mitra², T.H. Foster²¹Department of Physics, University of Rochester, Rochester, NY, USA; ²Department of Radiology, University of Rochester, Rochester, NY, USA

The availability of oxygen (³O₂) during photodynamic therapy (PDT) is a critical factor in the formation of singlet oxygen (¹O₂), which is responsible for tumor destruction in PDT. We have developed a complete theoretical model for rigorously describing the spatial and temporal dynamics of O₂ consumption and transport phenomena *in vivo*. The Krogh cylinder geometry was adopted, and we have significantly improved our previous model (Foster *et al* 1991) by considering a time-dependent O₂ source and consumption rate, linking the O₂ concentration in the vessel to that within the tissue through the Hill equation, and incorporating photobleaching effects, non-uniform photosensitizer distribution, and the axial diffusion of O₂ from the arterial to the venous end of the capillary. An initial steady-state distribution of O₂ concentration was established using parameters such as capillary dimensions, intercapillary distance, blood-flow velocity, metabolic consumption rate, and the arterial oxygen concentration. Time-evolved O₂

distributions were obtained by solving two-dimensional diffusion equations with an alternating-direction implicit method, and the self-sensitized $^1\text{O}_2$ -mediated bleaching mechanism developed by Georgakoudi *et al* (1997) was used. Other bleaching mechanisms can be incorporated easily. The mathematical model allows us to test the importance of axial diffusion and blood flow in PDT and to compute the distribution of photodynamic dose under different treatment conditions. The simulations predict that at higher O_2 consumption rates the contributions of blood flow and axial diffusion become pronounced and need to be included in investigating the O_2 distributions in the capillaries and the adjacent tumor tissue. Blood-flow velocity response to PDT and the dynamic variation of hemoglobin O_2 saturation within vessels under various irradiation conditions and with several different photosensitizers will be presented. Supported by NIH Grant CA 68409.

PII47

Preclinical evaluation of photodynamic therapy in new retinoblastoma xenografts

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Background: retinoblastoma is the most frequent ocular tumour in childhood. The treatment of patients with a germline mutation of RB1 gene is a challenge to achieve cancer cure, vision preservation and decrease of the risk of second malignancies. In this context, PDT appears as an alternative non mutagenic therapy.

The *in vivo* efficacy of PDT, was tested with 3 photosensitizers (mTHPC, verteporfin, hypericin) in 2 new human retinoblastoma xenografts in nude mice.

Material and methods: mice were separated into 4 groups (5-8 animals)(control, illuminated, PS and PS -illuminated groups). Hypericin antitumor effects were tested only in two groups (illuminated, PS-illuminated groups). Laser light (m-THPC, 75 J/cm², 514 nm, 100 mW; verteporfin, 50 J/cm², 692 nm, 600 mW), white light (hypericin, 75 J/cm², 75 W) were delivered to the tumors after drug injection (a.i): m-THPC, 0.3 mg/kg, i.p, 24 and 48h a.i; verteporfin, 1 mg/kg, i.v, 1h a.i; hypericin, 5mg/kg, i.p, 6h a.i.. m-THPC injections and illuminations were repeated 3 times.

Results: no phototoxicity nor toxicity was observed. PS-light combination always induced a tumor growth inhibition higher than PS alone. Hypericin and verteporfin had higher antitumor activity than m-THPC. Tumor growth inhibition was transient after one irradiation but growth delay was prolonged in the subsequent cycles of m-THPC + light.

Conclusions: these experimental data indicate that PDT did not show acute toxicity in mice and could efficiently slow down retinoblastoma growth. PDT might represent an interesting non mutagenic therapy.

PII48

Synthesis and preclinical studies of targeted, two-photon activated photo-dynamic therapy agents

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Photo-dynamic therapy (PDT) sensitizers that could be activated in the tissue transparency window (700-1000 nm) would circumvent the major limitation of poor tissue depth penetration common to currently approved agents. Single photon activation in this area of the spectrum becomes progressively more problematic as the activating wavelength increases. Two-photon activation does not exhibit this drawback, and we have synthesized a series of compounds with 2-photon cross sections large enough to be

considered for therapeutic PDT. We demonstrated 2-photon excited fluorescence for these compounds at depths of 3 cm in tissue phantoms, and efficient tumor cell killing down to 4 cm in collagen I phantoms. In addition to 2-photon activation in the tissue transparency window, our agents are targeted to the tumor tissue, and possess a module for near infrared fluorescence (IR) imaging. Using *in vivo* near IR fluorescence imaging of SCID mice, our compounds were shown to be targeted by the octreotate peptide to somatostatin 2 (SST2) receptors on MDA-MB-231 human breast cancer xenografts. Specificity of targeted uptake was determined by comparing uptake in MDA-MB-231 cells with uptake in Capan-1 SST2 receptor negative pancreatic carcinoma cells. Drug clearance for the targeted compound in tumor bearing mice was also imaged, and indicated that there would be a good window of time for therapeutic activation of the compound. We adapted a motorized X,Y,Z stage to raster a tumor bearing mouse under a powerful pulsed near I.R. laser, and preliminary studies using this system demonstrated regression of the tumor after treatment. Supported by Rasiris Inc. and the Montana Board of Research, Commercialization and Technology.

PII49

Photodynamic therapy for treatment of COPD. Clinical results

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Photodynamic therapy (PDT) is a minimally invasive treatment with great promise in malignant and non-malignant diseases. PDT with endoscopic delivery of light to hollow structures, has been successful in the treatment of a purulent bronchitis, empyema of a pleural cavity, duodenal ulcers and another non-oncologic diseases.

As the treatment of exacerbations of COPD is insufficiently effective now, we have applied in complex treatment of exacerbations of this disease a PDT-method. It is known, that some variants of PDT can render antimicrobial effect, and also intercept synthesis of proinflammatory cytokines by some cells and brake migration of fibroblasts and smooth muscle cells.

Group of the patients (45 patients) during treatment of an everyone exacerbation of COPD received local PDT. Photosensitizer "Photosens"(Russia) (sulphurized aluminium phthalocyanine) was delivered in bronchial tree of the patients through a ultrasonic inhaler of an original construction.

Laser action was carried out through a fiberoptic bronchoscope by diode laser "Kristall" (Russia) with a wavelength of a 670 nanometer and power 0,4-0,5 Watts. Light dose was be 7-12 joules on square centimeter of a mucosa of a bronchial tree (low-dose PDT). Control group (45 patients) received only conventional medicinal therapy.

Overseeing by the basic and control group of the patients was prolonged 3 years. The first clinical results have shown, that at the patients receiving PDT, a remission diseases began on 20 % earlier. The prompt positive changes proved to be true by examination of cell composition of a bronchial fluid and clinical observations.

The major interest represents overseeing in the main and control group of the patients in flow 3 years. The observations have shown, that quantity of exacerbations of COPD in year in group, receiving PDT, averaged 2, in control group - 4. The examinations of parameters of an external respiration (Vital lungs capacity, VC, Forced expiration for 1 second, FEV1, and Peak stream of an expiration, PEF) for 3 years in group, receiving PDT have shown, that the parameters of function of an external respiration have changed a little. In group of the patients receiving conventional therapy, the parameters of an external respiration became with more poor in full according to the prevailing concept of COPD pathogenesis.

The obtained results show, that the PDT-method in some cases can be used for the prevention of development of a pneumofibrosis.

PII50**Peripheral benzodiazepine receptors and apoptotic response in REH-cells after ALA-PDT**

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Recently attention has been focused on the involvement of the peripheral benzodiazepine receptors (PBR) in regulation of cell proliferative processes. Several studies have demonstrated increased binding site densities for PBR ligands in various malignancies. Due to the specific localization of PBR in the outer mitochondrial membrane, real possibility exists to influence the efficiency of aminolevulinic acid based photodynamic therapy by changing for instance outcome of apoptosis.

Thus, the aim of our study was to find out what is the role of PBR in the cellular response to ALA-PDT. Thus, in our experiments we tried to evaluate the modification of cell growth after ALA-PDT in Reh (B-leukemia) cells, when ligands of PBRs were presented in the medium. Data presented reveal, that cell growth evaluated 24 hours after treatment (ALA-PDT) in the presence of PBRs ligands was not modified by them. Other interesting question arises, whether presence of PBR ligands, following arrest of cell proliferation might be a result of induced apoptosis in Reh cells. Data obtained indicate, that increasing concentration of Pk-11195 might increase the yield of apoptosis up to 40%, whereas Ro5-4864 – just 10%. In contrary, ligands of central action – flumazenil and clonazepam – do not modify so much the growth of cell as ligands of peripheral action. In conclusion, ligands of peripheral benzodiazepine receptors might slightly modify the Reh cell response to ALA-PDT treatment. Particularly ligand Pk-11195 is able to diminish apoptotic cell response to ALA-PDT.

PII51**Spectroscopic monitoring during ALA-PDT of human basal cell carcinoma**W.J. Cottrell¹, T.H. Foster^{1,2}, A.R. Oseroff³

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Photodynamic therapy (PDT) using 5-aminolevulinic acid (ALA) is an effective therapy for treating basal cell carcinomas (BCC), with patients demonstrating high lesion clearance and excellent cosmetic outcomes. Treatment optimization promises improved efficacy and comfort for patients. In particular, high irradiance ALA-PDT is painful and may deplete tissue oxygen, but lowering the irradiance requires increased treatment time, which may be prohibitive clinically. Using compact instrumentation of our own design and construction, PDT dose metrics such as sensitizer photobleaching and hemoglobin oxygen saturation were monitored during ALA-PDT treatment of BCC as part of a study designed to guide treatment fluence and fluence rates in a pending clinical trial that will investigate optimized use of lower fluence rates. Fluorescence spectroscopy, corrected for the effects of tissue optical properties, reported PpIX photobleaching, photoproduct formation, and accumulation of endogenous porphyrins in the primary lesion and in the perilesion margin. Similarly, reflectance spectroscopy reported blood volume and hemoglobin oxygen saturation and was also used to generate corrections to the fluorescence spectra. The instrumentation controlled PDT treatment at 633nm while simultaneously monitoring fluorescence from 650 to 800nm. During a series of brief treatment interruptions at programmable time points, white light reflectance spectra and 405nm-induced fluorescence spectra between 430 and 800nm were acquired. Fluorescence spectra were decomposed into known fluorophore contributions using a robust singular value decomposition fitting routine performed in real time. Supported by NIH Grant P01 CA55719.

PII52**ALA-PDT attenuates expression of chimeric oncoprotein BCR-ABL in chronic myelogenous leukemia cells K562 and disrupts the cytoskeleton structure**M. Pluskalová¹, D. Grebeňová¹, K. Kuželová¹, P. Halada², Z. Hrkal¹¹Institute of Hematology and Blood Transfusion, Prague, Czech Republic; ²Institute of Microbiology AS CR, Prague, Czech Republic

Chronic myelogenous leukemia (CML) is the malignancy of hematopoietic stem cell which is characterized by the presence of an oncogene bcr-abl in hematopoietic precursors causing premature release of immature cells into the blood stream.

As the model for the studies of ALA-PDT cytotoxic effects on CML cells we employed CML-derived cell line K562 that expresses high levels of chimeric oncoprotein BCR-ABL. The deregulated (permanent) kinase activity of BCR-ABL leads to continuous proliferation of K562 cells and their resistance to the apoptosis promotion by conventional drugs. In contrast, ALA-PDT suppressed proliferation of K562 cells, which process was paralleled by an intermediate state resembling the late stage apoptosis and followed by the cell necrosis. The level of BCR-ABL protein decreased substantially within one hour after ALA-PDT due to the perturbation of HSP90/p23 chaperone complex of which the BCR-ABL is the client protein, resulting presumably in BCR-ABL proteasomal degradation. The proteomic analysis (two-dimensional electrophoresis in combination with MALDI-TOF mass spectrometry) revealed five proteins which were affected by ALA-PDT. Of them the PDZ-LIM protein CLP36 and cofilin play a role in the cytoskeleton organization. Another cytoskeleton-associated protein Septin2, the mobility of which was altered in response to ALA-PDT, participates in stabilization of actin stress fibers. To determine whether ALA-PDT affected the cytoskeleton structure we employed FITC-Phalloidin staining of polymerized actin and fluorescence-microscopy observation or flow cytometric analysis of stained cells. Contrary to uniformly stained actin filaments in control cells, the polymerized actin appeared as clusters in treated cells. Flow cytometry assay revealed that only 41 ± 6 percent cells had intact cytoskeleton two hours after ALA-PDT which number further decreased to 6 ± 1 % within next twenty hours, also reflecting cytoskeleton disruption induced by ALA-PDT.

We conclude that suppression of BCR-ABL caused by ALA-PDT leads to the dephosphorylation of cofilin resulting in actin depolymerization, collapse of cytoskeleton structure and to the cell necrosis.

The work was supported by grant NL 7681-3 of Internal Grant Agency, Ministry of Health, Czech Republic.

PII53**Photodynamic treatment with 5-aminolevulinic acid induces mitotic arrest in HeLa cells**

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5-aminolevulinic (ALA) is nowadays being widely used in Dermatology for diagnosis and treatment of skin lesions by using photodynamic therapy. We have studied the effects induced by 1 mM ALA on the human HeLa carcinoma cell line, employing different incubation (ranging from 2 to 6 h) and irradiation times (2-10 min) with blue light (3.4 mW/cm²). No dark cytotoxicity was detected by the MTT assay in cells only treated with ALA. However, HeLa cells incubated with ALA and subjected to light irradiation exhibited a variable damage regarding to cell viability, which was dependent on both incubation and irradiation times. Under sublethal experimental conditions, a clear increase in the mitotic index (MI) revealed by immunofluorescence detection of α -tubulin was observed. Values of 2-3 fold over the normal MI of HeLa cells (4.2 %) were scored. The increase in MI was due to a metaphase arrest induced by the photodynamic treatment. Many of

the metaphase cells showed altered mitotic spindles and, additionally, not all the chromosomes were well organized at the equatorial plate as detected by Hoechst-33258 staining. The implications of the mitotic arrest in cell death by apoptosis is being analysed.

PII54

Ultraviolet-induced autofluorescence characterization of normal and tumoral esophageal epithelium cells

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The cellular autofluorescence was characterized in normal human esophageal cells and in malignant esophageal epithelial cells. The study was performed under excitation at 351 nm where the cell fluorescence is mainly due to the reduced pyridine nucleotides (NAD(P)H) with a very small contribution from the oxidized flavins (FMN, FAD) or lipopigments. The results obtained by microspectrofluorimetry on monolayers or by spectrofluorimetry on cell suspensions were in good agreement: spectral distribution was very similar for squamous cell carcinoma, adenocarcinoma on Barrett's mucosa and normal cells. The relative contribution of each fluorophore to the fluorescence emission of the different cell types was evaluated by a curve-fitting analysis. A statistically highly significant difference was observed between the average intensity of the raw spectra of the different cell types. Tumoral cells had a fluorescence intensity approximately twice higher than that of normal cells. The nucleus autofluorescence showed the same spectral shape as that of the cytoplasm but with a lower intensity. The results of the NAD(P)H quantitation analyzed by microspectrofluorimetry on single living cells and spectrofluorimetry on cell suspensions were consistent with those obtained by biochemical cycling assay.

PII55

Combined resonance Raman and absorption microspectroscopy of single living erythrocytes underline the extreme photosensitivity of oxyhemoglobin

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Resonance Raman (RR) spectroscopy was performed on single living red blood cell (RBC) under 413.1 nm excitation, which induces selective enhancement of the haem vibrations.

We compared the sensitivity of oxygenated and deoxygenated RBC to repeated low power laser exposure ($P = 25 \mu\text{W}$, illumination time $t = 1 \text{ s}$). The most prominent band (ν_4) for deoxyRBC is always located at 1359 cm^{-1} , in good agreement with previous studiesⁱ⁻ⁱⁱⁱ. However, for oxyRBC, ν_4 originally appears at 1359 cm^{-1} and shows a progressive decrease after repeated exposure, along with the increase of a band located at 1376 cm^{-1} . This is in contradiction with the previous studies which have presented the ν_4 band for oxyhemoglobin to be located at 1376 cm^{-1} , as for methemoglobin.

We performed absorption spectra on the exact erythrocytes analyzed by RR spectroscopy, to confirm their oxidation state. The maxima of oxy-, deoxy- and met-RBC absorbance spectrum, for non-illuminated RBC, are correctly found at 415, 430 and 409 nm respectively. A significant blue shift was observed on the absorbance spectrum of oxyRBC, after RR spectral acquisition. This decisively shows that the ν_4 band for oxyhemoglobin is located at 1359 cm^{-1} . Photoconversion into methemoglobin results in a band shift to 1376 cm^{-1} .

We have demonstrated that oxygenated erythrocytes are very sensitive to laser exposure, which induces the degradation of oxyhemoglobin into methemoglobin. Deoxygenated erythrocytes

do not show such photosensitivity. The coincidence of the oxidation marker band ν_4 in oxy- and deoxyhemoglobin is in good agreement with the oxidation state of both compounds as Fe^{2+} .

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PII56

In vivo measurement of mTHPBC pharmacokinetic by using elastic scattering spectroscopy (ESS) in an improved xenografted athymic rat model with hepatic metastasis of human colon adenocarcinoma

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5,10,15,20-tetrakis(m-hydroxyphenyl)bacteriochlorin (mTHPBC) is a second generation photosensitizer with a strong absorbance peak at 734 nm that could be interesting for photodynamic therapy (PDT) of tumors located in highly pigmented tissues as liver. In the first step, a reliable pre-clinical model was developed in athymic rat. Before subcapsular injection of colon adenocarcinoma HT29 cells in the liver, immuno-suppression in the athymic rat was completed by a daily treatment of cyclosporine A (CyA, 20 mg/kg per day, 4 weeks) or by a 3.5 Gy whole body irradiation. Third group included the animals without pre-treatment. Among three tested groups the best tumor take rate was observed in pre-irradiated group (74 %) with a shortest delay of tumor appearance (72 ± 13 days) and highest tumor volume of 2949 mm^3 .

The diffuse reflectance spectra intensity of mTHPBC (D) was measured by ESS in the liver tissue from 2 to 24 hrs after intravenous injection of mTHPBC. These data were compared with mTHPBC intratissular concentrations (C) obtained from spectrofluorimetry data after liver resection and chemical extraction of the hepatic tissue. The pharmacokinetic profiles obtained by the two methods showed an excellent correlation ($R=0.997$, $C/D = 1.4 \pm 0.7$). In normal liver mTHPBC concentration was maximum at 4 hrs after injection ($4.54 \pm 1.56 \mu\text{g/g}$ tissue vs 0.3 ± 0.1 D) then progressively decreased until 24 hrs ($0.53 \pm 0.49 \mu\text{g/g}$ tissue vs 0.04 ± 0.04 D). In tumors, we observed the same pharmacokinetics profile, with mTHPBC of $0.86 \pm 0.5 \mu\text{g/g}$ tissue vs 0.06 ± 0.06 D at 4 hrs and $0.31 \mu\text{g/g}$ tissue vs 0.04 ± 0.02 OD at 24 hrs after injection. In conclusion, ESS is a non-invasive technique, which can successfully be used for pharmacokinetic measurements in liver with a sensitivity comparable to chemical extraction.

PII57

Rat neocerebellum cortex during normal and injured developing: an autofluorescence study

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The cerebellum cortex exhibits a multilayered tissue organization, undergoing sequential physiological, molecular and morphological changes during postnatal development, thus providing a suitable biological model of tissue structural evolution. Since the whole tissue autofluorescence depends on the nature and intratissular arrangement of histological components, tissue morphofunctional changes occurring during development are expected to modify autofluorescence properties. In this work a characterization of autofluorescence emission was performed on cerebellum cortex at different development phases, both under normal conditions and after mild injuries altering neocerebellar lobules development, induced by the cytostatic cisplatin. Rats at 11, 17, 30 Postnatal Days (PD) were used as a model. Autofluorescence signals (exc. 366 nm) were analyzed via fiber-optic probe at the cortex surface, as bulk tissue, and by microspectrofluorimetry on frozen tissue sections. Autofluorescence intensities (a.u.) measured in bulk tissue at PD11, PD17 and PD30 were 105, 137 and 85 in the control and 94, 117 and 118 in the treated neocerebellum, respectively. These

results are consistent with the changes of autofluorescence topological distribution occurring during normal and injured development, as verified on tissue sections through microspectrofluorometric analysis. When compared to untreated rats, treated ones exhibited a broadening towards longer wavelengths of the spectral shape. In treated rats, spectral fitting analysis evidenced an increase in flavins and lipopigments autofluorescence contribution, consistent with a general increase in oxidized state.

PII58

Optical pharmacokinetics of photosensitiser aluminium disulphonated phthalocyanine

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The ability to non-invasively measure the concentration of a photosensitising drug in tissues could provide substantial benefits for photodynamic therapy (PDT). For instance, provided that the technique can produce real-time data, it could be used to determine the concentration of the photosensitising agent just prior to light dose delivery and thereby optimise treatment efficiency.

The emphasis of this study is to investigate the potential of optical pharmacokinetics (OP) to provide a quantitative evaluation of the photosensitiser aluminium disulphonated phthalocyanine (AlS₂Pc) in tissue. The principle of OP relies on Elastic Scattering Spectroscopy (ESS), using a 2-fibre probe one of which delivers white light to the targeted tissue and the another one collects the scattered light.

AlS₂Pc was administered *via* the tail vein of Wistar rats. At various time intervals (1 h to 31 days), diffuse reflectance spectra were collected in the liver *in vivo*. Photosensitiser concentrations were then estimated using three spectral features: (i) intensity of the absorption peak, (ii) surface area under absorption band, and (iii) logarithm ESS spectra attenuation. For comparison sensitiser concentrations were also measured using spectrofluorimetry after alkaline extraction.

The OP values were found to mirror the concentration of AlS₂Pc measured after chemical extraction. The optimal way of analysing the spectral data, however, remains under discussion.

Similar studies are under way on other organs and further work is still necessary before this method can be validated. Nevertheless, this preliminary data illustrates that optical pharmacokinetics could offer the dual advantage over traditional methods in that it can be carried out *in vivo* and that real-time results can be generated.

PII59

Selective accumulation and photobleaching of indocyanine green in tumors measured by fluorescence and absorption spectroscopy

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Indocyanine green (ICG) is widely applied in medicine as an angiographic agent. It has absorption band in near infra red (NIR) with maximum about 800 nm and fluorescence emission maximum at 830 nm. Recently it has been claimed that this dye can be applied as photodynamic agent. Its advantage of absorbing in NIR suggests possible application of ICG for therapy of pigmented lesions such as melanoma or aged related macular degeneration (AMD).

In this work we studied the accumulation dynamics of ICG in mice with inoculated tumors after system administration. The absorption and fluorescence spectroscopy *in vivo* were applied to evaluate ICG concentration at different time intervals after input in tumor and normal muscle. Due to the fact that ICG absorption peak is in the tissue transparency band its contribution into total tissue absorption can be easily separated. We applied dye doses in the range of 10-40 mg/kg and measured tissue absorption and fluorescence spectra at

different time intervals up to 5 days after input. It was found out that dye concentration in tumor was 4-5 times more than in normal tissues in 24 hours after injection suggesting that this dye-light interval would be more preferable for light inactivation of dye. The ICG has low photostability in tissues and we found out that light doses of 300 J/cm² resulted in complete dye photobleaching in tumors. The light irradiation of tumors resulted in tumor deoxygenation. One day after irradiation the irradiated spot was necrotized. We assume that both mechanisms photodynamic and photothermal may be involved in the effect observed.

PII60

Developing fluorescence probes for reactive oxygen species detection

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For several years, growing interest has been given to understanding the role of reactive oxygen species (ROS) in pathological situations. Indeed, ROS seem to play a pivotal role in many diseases such as inflammatory processes, DNA damages, aging, cancer, ischemia-reperfusion injury. Thus, in order to clarify their way of formation, their exact implications in pathology as well as the nature of the cell component they affected, it is important to be able to detect them.

Among the several techniques available, fluorescence remains the most convenient and the most sensitive. For that purpose, different fluorescent probes were developed. These probes consist of a non-fluorescent molecule, which becomes fluorescent after the interaction with a ROS. However, they frequently suffer a lack of sufficient selectivity.

Recently, K.-I. Setsukinia et al. (J. Biol. Chem., 2003, 278(5), 3170-3175) developed a new fluorescent probe, 2-[6-(4'-hydroxy)phenoxy-3H-xanthen-3-on-9-yl]benzoic acid (HPF) able to release fluorescein by reacting with ROS. However, the mechanism of reaction of that probe was not well characterized.

Therefore, in order to design new fluorescent probes able to react specifically and efficiently with the different ROS produced in pathological conditions, we devoted the present study to assess the mechanism of reaction of the probe HPF with various ROS.

PII61

Depth-resolved fluorescence measurements of quantum dots in scattering medium

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Fluorescence spectroscopy nowadays is widely employed in many areas of biology and medicine, however measurements from deeper layers of the tissue are problematic due to a weak signal and light scattering. Luminescent quantum dots have been recently developed as an alternative to organic dyes and sensitizers for fluorescence based applications. An important component of the fluorescence spectroscopy is the geometry of the illumination and collection system. The use of multidistance probe allows to detect signals originating in the deeper layers and to measure the depth-resolved fluorescence in layered turbid medium.

The primary goal of this study was to characterize the spatially resolved fluorescence of quantum dots in turbid medium identifying the conditions of the fluorescence signal detection from the deeper layers.

The fiber optic probe, which consists of 8 collection fibers located at increasing distances (1 to 8 mm) from the single illumination fiber (irradiation with LED $\lambda_{em} = 470$ nm), was placed on the medium surface. The turbid media was modelled in the special cuvette with 10 compartments of 1 mm width each. First four were filled with milk-based scattering medium and the next four with (CdSe)ZnS quantum dots $\lambda_{em} = 537$ nm and $\lambda_{em} = 603$ nm respectively. The spatially resolved fluorescence was recorded to describe the

layers of quantum dots embedded at different depths within a homogeneous turbid medium.

The fluorescence signals demonstrated statistically significant difference detecting it at increasing distances from an illumination fiber. The fluorescence from deeper layers prevailed at higher distances. It was also possible to detect the change in thickness of fluorescing layers.

Such findings indicate that employed optical system could be used to visualize the changes in the tissue, such as accumulation of the sensitizer or thickening/ diminution of the inner layers of the skin, which sometimes can indicate the evolving of the tumour.

PII62

Comparison between HP- and GFP- mediated Fluorescence Reflectance Imaging of HELA tumor

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Green Fluorescent Protein (GFP) is used as a fluorescent marker for gene expression in a variety of organisms (ranging from bacteria to higher plants and animals) and for cells. The very bright GFP fluorescence enables internal tumors and metastases to be observed externally in critical organs such as colon, liver, bone, brain, etc., without contrast agents or other compounds or treatments.

With standard transfection methods we obtained a HELA cell line stably expressing GFP protein. We evaluated the capability of imaging HELA tumor cells both *in vitro* and *in vivo* on small animals by exploiting the GFP emission. The apparatus for the excitation and detection of GFP fluorescence is constituted by: a) 150 w lamp; b) excitation filter with a bandwidth centred at 488 nm; c) stereomicroscope equipped with a filter with a bandwidth centred at 508 nm and high sensitivity chilled CCD camera; d) Hamamatsu Wasabi acquisition and analysis system.

After the characterization of the HELA cells in terms of fluorescence yield, transfection rate and expression stability, they were injected in mice and FRI measurements were performed. The GFP mediated fluorescence imaging was compared with the Hematoporphyrin-FRI, by injecting mice with untransfected HELA cells and hematoporphyrin dichlorohydrate.

PII63

Porphyrim derivatives as photodiagnostic agents

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Porphyrim derivatives have been the subject of intensive studies because of their potential use as photosensitizers in the treatment of tumours. Photodynamic Therapy (PDT) plays already a landmark in the treatment of neoplastic lesionsⁱ. Porphyrim derivatives due to their good selectivity and uptake in cancer cellsⁱⁱ, are also promising compounds to be used in early diagnosis of tumours.

As a part of a program related with the development of new compounds with potential to be used as markers in the photodiagnosis of neoplastic tissues we report here the synthesis and the biological evaluation of new porphyrim derivatives coupled to

phenolic derivatives. The results and experimental procedures will be shown and discussed.

Acknowledgements: thanks are due to the University of Aveiro for funding the project "Neoplastic Photodiagnostic: new markers and biophysical properties" and to Science and Technology Foundation, FCT/FEDER for funding the Organic Chemistry Research Unit.

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PII64

Interactions of 3-aminobenzonitriles with human serum albumin studied by fluorescence spectroscopy

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The fluorescence properties of 3-aminobenzonitriles (3-ABNs) depend significantly on the surrounding environment. In hydrophobic solvents the fluorescence quantum yield and lifetime of 3-ABN are relatively large (0.18 and 2.4ns, respectively in *n*-hexane and 0.39 and 7.5ns, respectively in acetonitrile), while in water the fluorescence is quenched dramatically (0.002 and 0.045ns, respectively). Such unique photophysical properties of 3-ABN afford useful information on its microenvironment and can be used to develop new fluorescent probes. In this study, interactions of 3-aminobenzonitrile (3-ABN), 3-dimethylaminobenzonitrile (3-DMABN) and 3-diethylaminobenzonitrile (3-DEABN) with human serum albumin (HSA) were investigated by fluorescence spectroscopy to demonstrate the usefulness of 3-ABNs as a fluorescent probe.

All the three 3-ABNs (3-ABN, 3-DMABN, and 3-DEABN) exhibited very weak fluorescence in water ($\Phi_f = 0.002-0.004$), and their fluorescence lifetimes in water were obtained to be 45ps, 257ps, and 330ps, respectively. Upon addition of HSA ($1.0 \times 10^{-5} \text{M} - 8.0 \times 10^{-5} \text{M}$) to the aqueous solutions of the 3-ABNs, the fluorescence intensity and lifetime increased dramatically (e.g. 3-ABN in HSA gave a double exponential decay with lifetimes of 2.3ns (35%) and 9.0ns (65%)). Also the fluorescence bands were blue shifted remarkably. The fluorescence anisotropy (r) of 3-ABN, 3-DMABN, and 3-DEABN showed remarkable enhancement in HSA solutions ($r = 0.24, 0.14, \text{ and } 0.19$, respectively) as compared to those in water ($r = 0.03-0.09$), suggesting that the aminobenzonitriles are bound at rotationally restricted sites in HSA.

The unfolding behavior of HSA was examined by chemical denaturation with guanidine hydrochloride (GdHCl) as a denaturant. With increasing the concentration of GdHCl in the range of 0M to 5M, the fluorescence intensity and the mean lifetime of 3-DMABN and 3-DEABN gradually decreased until about 2M GdHCl and more significant drops were recognized between 2.0M and 4.0M. This suggests that HSA denatured by a two-stage process.

PII65

Fluorescent diagnostics in gynecological cancer with alasense

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Fluorescent diagnostics (FD) using Alasense (5-aminolevulinic acid, NIOPIC, Russia)(AS) as photosensitizer during standart laparoscopy have been provided in 10 patients with T1-4 stage ovarian cancer (OC) of different histology for evaluating the

completeness of response after previous complex therapy or in cases of suspicion of recurrence of disease. Previous complete clinical and instrumental investigation (ultrasound, computer tomography) showed no signs of peritoneal metastases or liquid in peritoneal cavity in these patients. FD was done in 20 patients with cancer of the cervix (CC) for detecting the borders of tumor growth. AS was given per os in dose 20 mg/kg of body weight in 150 ml of water solution. Laparoscopy in white light and FD with detecting of the fluorescence zones, borders of metastases dissemination and intensity of accumulation of AS in metastases of OC and adjusting tissues has been provided 4 hours after AS administration with Spectral-fluorescent Complex (He-Ne-laser, $\lambda = 633\text{nm}$) with CCD-camera. During FD in CC we used additionally light sources 380 nm. We've found intensive fluorescence zones on peritoneum in 9 patients, the number of zones in patients was from 1 till 7, the sizes of it from 0.2 till 0.9 cm. Biopsies were taken from all fluorescence zones for cytological and histological examination. Morphological verification of metastases of ovarian cancer has been got in 8 patients in all samples. We got fluorescence in all CC patients, tumor borders in 67% exceeded clinically detected ones. No skin phototoxicity and other side effects were found after AS administration or during laparoscopy with FD. Our experience show pronounced efficacy of laparoscopic FD with Alasense comparing to traditional one in white light for early evaluating of peritoneal dissemination of OC and in CC with high specificity and sensitivity.

PII66

Fluorescent diagnostics of oral cancer with Alasense

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The aim of the study was to work out the regimes of fluorescent diagnostics (FD) in oral cancer patients with photosensitizer 5-aminolaevulinic acid (Alasense, "NIOPIC", Russia) (AS), evaluate the toxicity of AS and determine the efficacy of FD with AS. FD with AS have been provided in 28 patients with T1-4 stage tumors (histology - squamous cell carcinoma (SCC)). Primary tumor sites were oral cavity and oropharynx. AS was given per os in dose 20 mg/kg of body weight in water solution. FD with detecting the borders of tumor growth and intensity of accumulation of AS in tumor and adjusting tissues has been provided 1,2,3 and 4 hours after AS administration with Spectral-fluorescent Complex (He-Ne-laser) with CCD-camera. The highest rate of accumulation of AS and fluorescent contrast was got 3 and 4 hours after administration of AS. Using light sources (380 – 440 nm) we've got the visual 2-dimensional picture of fluorescing zones. We've got fluorescence in all cases: in 68% of patients it exceeded the borders of clinically detected sites for 0.2 - 4.0 cm, fluorescent contrast between tumor and adjacent normal tissue was 2.2 – 5.9. In 4 patients additional fluorescence zones were found, cytological verification was got in all cases. No phototoxicity and other side effects were found during FD. FD is providing diagnostically significant information about disease advance, borders of tumor growth, often exceeding clinically detected sites, allowed identification of subclinical lesions in patients with primary multiple cancer and locally spread recurrences. FD demonstrated high sensitivity and specificity. Photosensitizer AS has high fluorescent activity and no significant toxicity with the exception of short-term increasing of sensitivity of skin to direct sunlight. Combined analysis of both fluorescent image and spectrometric data gives a possibility of improving of the results of FD in oral cancer patients.

PII67

Photoinactivation of wastewater microorganisms by cationic and neutral porphyrins

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It is well known that the high growth of the population in urban areas increases the amount of wastewater to treat. The lack of new technologies for the wastewater treatment induces the reduction of water resources, and increases the environmental pollution.

The possibility of using photosensitizers as a chemical free approach for killing pathogenic microorganisms and photodegradation of pollutants in water seems to be very promising. Although the transmission of microbiological diseases has been reduced by the development of good water supplies and hygienic-based procedures for a whole range of human activities, it is highly important to persist in the development of novel, convenient and inexpensive methods for combating microbial diseases¹.

In this communication we will describe the microbiological results obtained when cationic and neutral porphyrins were used in the directly photoinactivation sewage bacterial assemblages.

Acknowledgments: thanks are due to "Fundação para a Ciência e a Tecnologia, FCT/FEDER" and to the University of Aveiro for funding this work. J. P. C. Tomé also thanks FCT for a postdoctoral grant.

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PII68

In vitro activity against herpes simplex virus type I of beta cationic meso-tetraphenylporphyrins

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For the last two decades, interdisciplinary studies performed with porphyrin macrocycles pointed out the great potential of this type of compounds for applications in various fields as catalysis, advanced biomimetic models for photosynthesis, new electronic materials, sensors and drugs.¹ Concerning biomedical applications a number of porphyrinic compounds are already being used with success for the treatment of several diseases by photodynamic therapy, namely in the selective inactivation of microorganisms.¹¹

Following our interest in obtaining compounds with adequate physicochemical and biological properties for medicinal applications, we describe here an easy synthetic route to two cationic beta-vinyl substituted meso-tetraphenylporphyrins and preliminary studies showing their capacities in the photoinactivation of herpes simplex virus type 1 (HSV-1). The two derivatives were efficiently synthesized by stereospecific aldol-like condensation of 1,2- or 1,4-dimethylpyridinium iodide with 2-formyl-meso-tetraphenylporphyrin. The two compounds displayed similar photocytotoxicity profiles, however virus inactivation studies carried out with HSV-1 pointed out a striking difference between them. One of the compounds was able to photoinactivate

97% of the viral population, while the other isomer, under the same conditions, displayed no virucidal effect.

Acknowledgements: we gratefully acknowledge FCT/FEDER for funding (Project POCTI/38750/FCB/2001) and financial support of the Research Unit "Química Orgânica, Produtos Naturais e Agro-Alimentares". F. G. and J. P. C. T. are grateful to FCT for their post-doctoral grants. E. M. P. S., R. R. D., and I. N. P. are grateful to FCT for their BIC grants.

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PII69

Tetraaryl-porphyrins as antibacterial photosensitizers

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Multidrug-resistant pathogens are constantly increasing because of the overuse of antibiotics. Antimicrobial Photodynamic Therapy (PACT) provides a viable therapeutic option for the treatment of bacteria and it has recently found wide application either *in vivo* and *in vitro* studies.

It is known that Gram-negative bacteria are more resistant to antibiotic agents and to the combine effect of light and photosensitizer; furthermore it is known that cationic photosensitizers are more efficient than neutral or anionic ones.

In this work we present the results of the photodynamic inactivation of Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and of Gram-positive bacteria *Staphylococcus aureus* when illuminated in the presence of tetraaryl-porphyrins. The degree of photoinactivation has been studied with six different photosensitizers, two commercial compounds (**1**, **2**) and newly synthesized compounds, three positively charged (**3**, **4**, **5**) and one neutral (**6**) porphyrins.

The bacteria were incubated for 30 min with variable concentrations of photosensitizers in the range 0.1 – 50 µM, then irradiated for 60 min (Gram-negative) and 15 min (Gram-positive) with a 500 W tungsten-halogen lamp, fitted with a circulating refrigerated aqueous filter. The cell survival was determined by plate count technique on LB agar.

As expected, results indicate the Gram-positive bacteria is easily inactivated under the conditions reported, while the Gram-negative show higher resistance to the treatment; in particular, in the case of *E. coli* 0.01 % of cell survival was obtained with 0.4 µM with the most active molecule (**4**) and 18.0 µM with the lowest efficient compounds (**1**). When *P. aeruginosa* was treated with the photosensitizers, a comparable cell survival (0.1 %) was obtained in the range of 15 µM of the most effective photosensitizer (**3**) up to 50 µM for the less active compound (**6**).

PII70

The treatment of leishmaniasis using photodynamic therapy

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Leishmaniasis affects 12 Million people worldwide in 66 Old World and 22 New World countries; infection is found in 16 countries in Europe. Leishmaniasis is caused by parasitic protozoa of the family Trypanomatidae and genus *Leishmania*. The three diseases caused by this group include a disseminating visceral infection, and the more localised mucocutaneous and cutaneous infections. Humans are infected via the bite of female *phlebotominae* or *lutzomyia* sandflies. The parasites grow in the gut of the sandfly and are injected into the human when the vector feeds. There are few useful drugs for the treatment of the leishmaniasis and those drugs currently used, such as amphotericin

B, are highly toxic and costly with increasing reports of drug resistance.

Our work focuses on the development of porphyrins for use in the photodynamic therapy of localised Leishmania infections. Cationic porphyrins are currently being investigated for their anti-leishmanial activity by the group as they are thought to be attracted to the net negative charge of the lipopolysaccharides (LPS) associated with outer surface of the leishmanial cell. Proliferation assays of L.major promastigotes, macrophages and keratinocytes after PDT using four cationic porphyrins has shown selective differentiation between the three cell lines with LD90's as low as 1µM.

PII71

Photoactive pesticides for insect pest control: effects on leafminer *Liriomyza bryoniae* (Diptera, Agromyzidae)

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Development of new, ecologically inert technologies to control insect pest populations is of great importance for plant protection. In this context, photoactive compounds might be used as effective insecticide agents, having low impact on the environment, being nontoxic and not mutagenic. The point is, that photoactive compound accumulates within the insect and, following exposition to visible light, induces lethal photochemical reactions, e.g., damage of cuticle, Malpighian tubes, midgut wall, following feeding inhibition and death. Nevertheless, different insects have differential accumulation of photosensitizers, which are usually added to the bait. The aim of this study was to determine, whether polyphagous plant pest *Liriomyza bryoniae* (Diptera, Agromyzidae) is sensitive to photosensitization. For this purpose two different photosensitizers were used: hematoporphyrin dimethyl ether and methylene blue. Non-coherent light source producing visible light for excitation of corresponding photosensitizers was exploited.

Data obtained indicate, that survival of *Liriomyza bryoniae* adults might significantly decrease, when hematoporphyrin dimethyl ether was added to feeding bait and afterwards the flies were irradiated with visible light. Methylene blue was less effective. No effects on survival of flies were observed after exposure of flies to light alone or to bait, containing sensitizer.

PII72

Photosensitization action of some photosensitizers on whitefly (*Bemesia tabaci*)

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There are more than 1000 white fly species exist in the world. The sweet potato whitefly (*Bemesia tabaci*) is one of the most pestiferous of the group. It attacks more than 500 species of plants from 63 plant families. The damage caused by white fly are weakening and early wilting of the plant, reduction of the plant growth rate and yield. It may also cause leaf chlorosis, leaf withering, premature dropping of leaves and plant death. Honeydew produced by the whitefly serves as a substrate for the growth of black sooty mold on leaves and fruit. In addition to vectoring of plant viruses by this insect plant viruses transmitted by whiteflies cause over 40 diseases of vegetable and fiber crops worldwide. So, Hematoporphyrin (IX) possesses several favorable features for such application as photopesticides due to (1) they are endowed with a high quantum yield for generation of highly cytotoxic intermediates. (2) They absorb essentially all wavelengths in the sun emission spectrum, hence they display a large photoefficiency in field irradiations. (3) They typically act at the level of cell membranes minimizing the induction of mutagenic effects and the onset of photoresistance. (4) They have high

photosensitizing activity towards biological systems in addition; they are already approved for medical use in photodynamic therapy of tumors and other diseases. The efficacy of the used photosensitizers depends on the concentration, the fluence rate of irradiation and the exposure time. In this study we use natural sunlight as well as laser radiation with porphyrin derivatives as a novel technique for control whitefly (*Bemisia tabaci*) (Gennadius). The results obtained illustrated that the efficacy of hematoporphyrin against whitefly (*Bemisia tabaci*) varies from one concentration to another, the highest concentration, 3×10^{-3} mol dm⁻³ gives up to 73 % mortality while the lowest concentration, 10^{-6} mol dm⁻³ gives up to 36 % mortality. On the other hand the effect of laser on the whitefly fecundity, showed that the optimum dosage required for the radiosterilization of whitefly adults are (514.5 nm, 150 mW) for argon laser and (308 nm, 15 mW) for He-Cd laser. No hatching was observed in eggs laid by females irradiated by the two types of laser.

Key words: Haematoporphyrin, Laser, whitefly (*Bemisia tabaci*).

PII73

Late stages of photolysis: cone vs. rod visual pigments

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Late stages of the photolysis of visual pigments play a crucial role in recovery of photoreceptor sensitivity after bleaching. Ten times faster dark adaptation of cones as compared to rods may imply faster decay of the photolysis products of cone pigments. However, there is no data on the kinetics of photolysis of cone visual pigments *in situ*. Thus the aim of our work was to study late stages of the photolysis of visual pigments in intact cones and rods.

Visual pigments of goldfish rods and red cones were studied using fast-scanning dichroic microspectrophotometer. We found that the basic products of photolysis of the cone visual pigment are similar to those of rod porphyropsin but decay substantially faster. Immediately after fast bleaching metapigment II in equilibrium with metapigment I appears both in cones and rods. Further they decay to 3-dehydroretinal and opsin. However, no metapigment III can reliably be detected in goldfish photoreceptors. In cones, metapigment II decays to 3-dehydroretinal and opsin almost 90 times faster than in rods. Kinetic analysis of metaproducts' decay and 3-dehydroretinal to 3-dehydroretinol conversion indicates that the limiting stage in 3-dehydroretinol production in rods is the decay of metapigment II to 3-dehydroretinal and opsin while in cones the enzymatic reduction of 3-dehydroretinal is rate limiting. Together with previously obtained data on cone-like visual pigment of frog green rods (Golobokova et al., 2003) these results show that high rate of metapigment II decay is characteristic of cone visual pigments as opposed to rod rhodopsin/porphyropsin. The two features of cone visual pigments, that is fast quenching of the residual activity of cone metaproducts due to fast hydrolysis of 3-dehydroretinal/opsin Schiff base, and correspondingly fast appearance of the substrates for dark visual pigment regeneration (free opsin and 3-dehydroretinol) are essential conditions for faster dark adaptation of cones as compared to rods.

PII74

Light induced melatonin suppression – indications for a dose dependence

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Based on published data we discuss temporal aspects of light induced melatonin suppression. The aim of this work is to derive if the light induced melatonin suppression is "dose" (energy)-dependent or "intensity" (power) -dependent. The two terms are often mixed up and the practical importance of the time dependence of the melatonin suppression is often overlooked.

The Bunsen-Roscoe law of photobiology states that the effect is independent (within a certain general time frame) of the duration of exposure as long as the radiant exposure is the same. For a high irradiance level, only a short exposure duration is necessary to obtain a given level of radiant exposure and effect. For a low irradiance level, the exposure duration has to be correspondingly longer to evoke the same effect. Some experimental studies postulate a "dose"-dependency for light induced melatonin suppression studies, i.e. the effect is proportional to the "dose" (i.e. the radiant exposure, or the number of photons, independent of the time it took to receive these photons). Others only refer to the irradiance and not to the exposure duration thus implying that the effect, for a given irradiance level, does not depend on exposure duration.

By analysing the linear inverse relationship between reduction of melatonin concentration and duration of light exposure given the literature data, it seems that the effect of light induced melatonin suppression depends on the photon dose (photons/cm²) incident upon the retina. In a study conducted by McIntyre et al. a light exposure of 400 lux for 30 min lead to a melatonin reduction of $9 \cdot 10^{-12}$ g/ml, an exposure to 200 lux for 60 min lead to nearly the same reduction of $10 \cdot 10^{-12}$ g/ml. Study data from Smith et al. show that after a light exposure of one hour of the middle visual field (illuminance at the corneal level: 1000 lux) a reduction in melatonin of 21 % was observed, after two hours of light exposure the reduction was 42 %. Considering the results of these experimental studies it seems that the dose-response relationship for light induced melatonin suppression is valid for at least one hour.

The analysis of the data of the studies from Brainard et al and Thapan et al supports the assumption of the dose-dependence. When one compares only irradiance levels leading to a certain suppression effect without reference to exposure duration, the data do not compare well. When one takes the exposure duration into account and calculates the effective radiant exposure for the reported irradiances then the data compare well, with differences of less than 25 %.

PII75

Time series study in a freshwater lagoon of Patagonia: solar acclimation of phytoplankton measured by pulse amplitude modulated (PAM) techniques

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A time series study conducted during 2000-2002 at Chiquichano Lagoon (43°14'S, 65°18'W, Trelew, Argentina), determined a microplankton bloom ($>400 \mu\text{g chl-a l}^{-1}$) during summer. A new study was initiated in February 2005 to evaluate the impact of solar radiation on photochemical parameters of phytoplankton assemblages throughout the year. Samples are collected the evening before experimentation, pre-filtered to remove zooplankton ($>100 \mu\text{m}$), and kept in the dark (night time). In the samples collected so far, chl-a varied between 100 - 700 $\mu\text{g chl-a l}^{-1}$ and nanoplankton ($<20 \mu\text{m}$) accounted for 61 - 95%. Samples are dispensed in quartz tubes under different radiation treatments (duplicates): a) PAB (280-700 nm), uncovered tubes; b) PA (320-700 nm), tubes covered with a cut-off filter at 320 nm, and c) P (400-700 nm), tubes covered with cut-off filter at 395 nm. The tubes are exposed to solar radiation in a water-bath for temperature control. The effect of solar radiation on photosystem II is monitored throughout the day by measuring the photosynthetic quantum yield (Y) and other parameters with a Water-PAM fluorometer (Walz). Additionally, samples are taken at local noon and at the end of experiments (evening) to evaluate recovering in the dark. A significant decrease in Y was determined at high irradiances as compared to the initial values in all treatments, and the Y values at noon were $19\% \pm 12$, $24\% \pm 14$ and $42\% \pm 16$ of the initial in the PAB, PA and P

treatments, respectively. A significant recovery is observed when cells are transferred to dim light, but it is not complete, even after 20 hours, when *Y* reached $70\% \pm 22$; $74\% \pm 18$ and $81\% \pm 14$ of the initial value in the PAB, PA and P, respectively, thus indicating that some chronic damage persisted in the cells. The samples with high chl-a concentration, i.e., $> 400 \mu\text{g chl l}^{-1}$, were the most inhibited, suggesting a "dark" acclimation of these samples in an environment with high attenuation of solar radiation.

PII76

In situ variability in photosynthetic quantum yield in phytoplankton assemblages from a freshwater lagoon in Southern China

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During March-April 2005 we carried out studies to determine the daily variability in photosynthetic parameters in phytoplankton assemblages from a freshwater tropical lagoon in Southern China (23.3° N, 116.6° E). Water samples were taken every hour within the upper mixed layer at 0, 0.5 and 1 m depth. Additionally, a surface sample (taken at 8:30 am) was exposed to surface irradiance inside quartz tubes (i.e., fixed samples) under three radiation treatments (i.e., PAB: 280-700 nm; PA: 320-700 nm; and P: 400-400 nm). Photosynthetic parameters were measured hourly in the lagoon and in the fixed samples using a Water-PAM fluorometer (Walz). Solar radiation (UV-B, UV-A, and PAR) was continuously monitored with a broad-band filter radiometer (ELDONET), and attenuation in the water column was obtained with an ELDONET submersible radiometer; temperature was monitored hourly with a YSI 600XL probe. PAR irradiance at noon varied between 250 and 450 W m⁻², and the attenuation coefficient (k_{PAR}) was 1.2 m⁻¹. During the study period, chl-a concentration varied between 4 and 27 $\mu\text{g chl-a l}^{-1}$ and the community was dominated by of chlorophytes and cyanophytes. The initial quantum yield (*Y*) at the surface was variable between 0.3 and 0.6, mainly due to changes in the radiation conditions (i.e., sunny vs. cloudy days). There was a significant increase of *Y* with depth, suggesting that mixing was not strong and some differential acclimation occurred in the water column. A significant decrease in *Y* was determined at noon in all samples, but they fully or partially recovered in the afternoon. The fixed samples had the same daily pattern as those collected at different depths, and they were inhibited by UVR up to 75%. No significant differences were found between fixed samples exposed to PAR and the surface samples in the lagoon throughout the day, suggesting that the slow (but significant) natural mixing was important to dilute out the impact of UVR observed in the samples.

PII77

UV and VIS photodegradation of triazines and triazine derivatives – catalyzed and sensitized reactions

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Photolysis in UV region and photocatalyzed and photosensitized reactions in VIS region were studied with triazine herbicides atrazine, simazine, propazine and with atrazine metabolites desethylatrazine (DEA) and desisopropylatrazine (DIA) under laboratory conditions.

UV degradation of triazines and their metabolites is a direct photolytic reaction unaffected by pH or metal (ferric) ions. Rate constants of the photolytic degradation are of the same order of magnitude for all the substrates studied (from 2.3×10^{-4} for DIA to $4.7 \times 10^{-4} \text{ s}^{-1}$ for propazine), the pesticides are more reactive than their metabolites. Photolysis of all substrates were slowed down in natural humic waters, the decrease of the rate constant is

proportional to the increase of absorbance at 254 nm in humic water. Sensitizers producing singlet oxygen has no effect on UV degradation.

Degradation of atrazine, simazine and propazine in VIS region proceeds either as photocatalytic reaction (in the presence of Fe³⁺ ions) or as a photosensitized reaction (in the presence of sensitizer producing o_2^-). DEA and DIA were degraded only in the presence of photosensitizer. Atrazine is the most reactive substrate in photocatalyzed as well as in photosensitized reaction.

PII78

Combined effects of nutrient limitations and UV radiation on viability, metabolic activities and DNA damages in the marine *Vibrio angustum* S14

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In many aquatic environments bacteria are limited by nutrients (organic carbon, inorganic nitrogen or phosphorus) and there is now strong evidence that solar ultraviolet radiation (UVR) could affect bacterial activities in surface water. However little is known about the combined effects of nutrient limitations and UVR on bacterial growth and viability in aquatic ecosystems. We studied the effect of UVR on the marine *Vibrio angustum* S14 cultivated under different nutrient limitations (carbon, nitrogen, phosphorus). Cells were harvested in stationary phase and exposed to artificial solar radiation at 25°C. Bacterial viability and rapidity to regrowth in a new media were more severely affected in N- and P-depleted cultures after irradiation compared to C-depleted cultures, because of greater sensitivity to UVB radiation. Spectral actions for inhibition of DNA and protein synthesis were determined for cells exposed to a broad range of irradiance treatments by measuring 3H-thymidine and 3H-leucine uptake respectively. The majority of DNA synthesis inhibition was due to UVB radiation, while protein synthesis was affected both by UVB and UVA radiation. DNA and protein synthesis were more severely inhibited in N- and P-depleted cultures but spectral actions for inhibition of both synthesis activities were only slightly modified by these limitations. The formation of the main DNA dimeric pyrimidine lesions was also studied using a HPLC-tandem mass spectrometry assay after 3 and 6h of exposure to artificial sunlight to determine if the nature of limitations modified the induction of these damages in bacterial cells.

PII79

Interactive effect of nutrient concentration and ultraviolet radiation on three marine phytoplankton species

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Long term experiments (7 days) were conducted with three marine phytoplankton species - *Thalassiosira fluviatilis*, *Heterocapsa triquetra* and *Prorocentrum micans* - using both a temperature controlled (20° C) illuminated chamber (12:12 L:D cycle, 300 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for PAR, 15 Wm⁻² for UV-A, and 0.7 Wm⁻² for UV-B), and solar radiation during the austral summer. The cultures were grown in 4-liters UV-transparent Plexyglas containers and under at least 2 nutrient concentrations (natural seawater concentration and addition of 0.6 and 0.7 mM of NaPO₄H₂ and NaNO₃, respectively) and under 3 radiation treatments (PAB, 280-700 nm; PA, 320-700 nm; and P, 400-700 nm). Samples were taken every day at three times (8am, 1pm and 5pm) and photosynthetic parameters were measured with a Water PAM fluorometer (Walz). In addition, samples were taken for chl-a, and absorption

characteristics determinations as well as for cell counts. *T. fluviatilis* was the most sensitive species to UVR, with cell concentration decreasing with time in samples exposed to UVR. The photosynthetic quantum yield (Y) decreased significantly during the day in all treatments, although recovery was observed during the night. *H. triquetra* and *P. micans* were more resistant to UVR, and even though Y decreased significantly at local noon, cells recovered during the day. In both species, local noon inhibition decreased with time thus indicating an acclimation to solar UVR. This acclimation seems to be attained through the synthesis of UV-absorbing compounds that significantly increased throughout the experiment. Samples exposed to UVR and with higher nutrient concentrations were those that had the higher amount of UV-absorbing compounds.

PII80

Solar UV radiation modulates daily production and DNA damage of marine bacterioplankton from a productive upwelling zone (36°S), Chile

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The impacts of solar radiation on bacterioplankton secondary production (BSP) in upwelling ecosystems have significant implications for carbon cycling. In this context, we studied the daily effect of solar radiation (UVR and PAR) on BSP and the DNA damage-repair response by the cyclobutane-pyrimidine-dimmers (CPDs) formation. Experiments were conducted with two natural bacterioplankton assemblages (0.2-0.7 μm) collected off central-south Chile, during October-November, 2004. Surface (0.5 m) and subsurface water samples (80 m) were exposed to differential solar radiation using filters (i.e., PAB, 280-700 nm; PA, 320-700 nm; and P, 400-700 nm) during 5-20 h. Throughout 20 hours we estimated BSP (radiolabeled thymidine and leucine) and CPDs accumulation (immunoassay techniques). BSP was mainly affected by PAR in both assemblages, during high irradiance periods, followed by UVA and UVB respectively. Under full solar radiation and maximum irradiance period (around noon) surface waters had a maximum inhibition of BSP (78%) as well as low growth rates (μ) and efficiency (BGE). Subsurface assemblages on the contrary, showed an enhancement of BSP (205%), μ and BGE. Both bacterioplankton assemblages had a rapid accumulation of CPDs (60 CPDs Mb⁻¹) during high irradiance periods. However, BSP inhibition and DNA damage in surface assemblages were recovered after the overnight incubation reaching pre-exposure levels. BSP of subsurface assemblages was enhanced 25 fold through the afternoon and overnight incubation, despite that residual damage was detected at the end of the experiments (20 CPDs Mb⁻¹). These results indicate that surface and deep bacterial assemblages in this upwelling system are highly sensitive to solar radiation but respond in opposite ways to CPDs accumulation. BSP can be more inhibited in surface assemblages than in deep assemblages, but surface bacteria have more effective photo-dark repair mechanisms.

PII81

Lack of reddening in Lake Tovel (Brenta Dolomites, Trento, Italy): photobiological aspects

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During summer large algal blooms were responsible of a spectacular water reddening in Lake Tovel in the Brenta Dolomites (Trento, Italy); this phenomenon has however no longer been observed since 1964. Previous studies had hypothesized that the green and red “forms” of the dinoflagellates involved in the bloom were two ecophysiological stages of the same species, linked by massive carotenogenesis triggered by high irradiance and nitrogen depletion (Baldi, *Mem. Mus. St. Nat. Ven. Trid.*, 6: 1-297, 1941).

In 2001 a research project (SALTO) has been funded by the local administration (Autonomous Province of Trento, Scientific Research Service) with the aim to study the environmental factors in the Lake Tovel area, the biology of the dinoflagellates involved in the red blooms and the causes of the ceasing of the reddening. To test Baldi’s hypothesis, enclosure studies were carried out to ascertain the physical and ecological aspects (such as light, competition, grazing, vertical migration) and the nutritional requirements for triggering the carotenogenesis and the blooming. Photosynthetic activity of the phytoplankton community in the enclosures was monitored, by measuring the optimal quantum yield of chlorophyll fluorescence F_v/F_m with a pulse amplitude modulation fluorometer (PAM). A massive algal vertical migration to the lake surface driven by positive phototaxis has been observed in the enclosures in sunny days. The possible effects of UV radiation on the blooming were tested either by removing or slightly increasing it by means of filters or fluorescent lamps, respectively. However no UV effect was observed in accordance with recent findings demonstrating that green and red “forms”, in spite of similar morphology, belong to different species and genera (Flaim *et al.*, *Phycologia*, 43: 737-743, 2004).

PII82

Spectroscopic studies on photocycle of the flavin-binding photoreceptor AppA, a bacterial transcriptional anti-repressor of photosynthesis genes

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The flavoprotein AppA from *Rhodobacter sphaeroides* contains an N-terminal domain belonging to a new class of photoreceptors designated as BLUF domains. AppA was shown to control photosynthesis gene expression in response to blue light and oxygen tension. We have investigated the photocycle of the AppA BLUF domain by ultrafast fluorescence, femtosecond transient absorption and nanosecond flash photolysis spectroscopy. Time-resolved fluorescence experiments revealed four components of flavin adenine dinucleotide (FAD) excited-state decay, with lifetimes of 25 ps, 150 ps, 670 ps and 3.8 ns. Ultrafast transient absorption spectroscopy revealed rapid internal conversion and vibrational cooling processes on excited FAD with time constants of 250 fs and 1.2 ps, and a multi-exponential decay with effective time constants of 90 ps, 590 ps and 2.7 ns. Concomitant with the decay of excited FAD, the rise of a species with a narrow absorption-difference band near 495 nm was detected which spectrally resembles the long-living signaling state of AppA. The nanosecond flash photolysis measurements indicated that formation of the signaling state was complete in 10 ns, and shows no further changes up to 15 μs . The quantum yield of the signaling state formation was determined at 24%. Thus, the signaling state of the AppA BLUF domain is formed on the ultrafast timescale directly from the FAD singlet excited state, without any apparent intermediate, and remains stable over 12 decades of time. In

parallel with the signaling state, the FAD triplet state is formed from the FAD singlet excited state at 9% efficiency as a side reaction of the AppA photocycle.

We have also performed femtosecond transient absorption spectroscopy on site-directed AppA mutants. The results of these photoinactive mutants suggest a transient formation of neutral semiquinones in about 20 ps, which decay on a timescale of ~1 ns.

PII83

A blue-light sensing, phototropin-related protein from *Pseudomonas putida*: a paradigm for an extended LOV construct

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 The *Pseudomonas putida* KT2440 gene PP2739 encodes a 151 amino acids protein (Q88JB0) with significant sequence similarity to the LOV (light, oxygen, voltage) domains of the blue-light sensitive protein YtvA from *Bacillus subtilis* and to plant phototropins (phot). This sensory box LOV protein (PpSB2-LOV), consisting of a LOV core, followed by a short C-terminal extension predicted to build an α -helix (analogous to the interdomain linker in YtvA), thus constituting a naturally occurring paradigm for an extended LOV construct. The recombinant PpSB2-LOV-protein shows a photochemistry similar to that of YtvA and phot-LOV domains. Furthermore, the *P. putida* sensory box protein contains a single tryptophan residue (W97), conserved within YtvA (W103) and phototropin LOV domains. The fluorescence spectrum and lifetime of this conserved tryptophan residue (W97) resembles the corresponding W103 in full-length YtvA; however, the fluorescence of W97 increases upon photoproduct formation, indicating larger light-driven conformational changes compared to YtvA. Therefore, it is concluded that the α -helical segment located at the C-terminus of the LOV core is sufficient to obtain comparable fluorescence characteristics of the conserved tryptophan residue in PpSB2-LOV and full-length YtvA. These results suggest that the mechanism of transmitting light-induced conformational changes to the effector domains is similar in bacterial LOV proteins and plant phot. Furthermore, they highlight the importance of the linker region in determining the interdomain interactions in this type of light sensors.

PII84

A flavoprotein isolated from phototactic zoospores of a brown alga, *Scytosiphon lomentaria*: a new member of “Old Yellow Enzyme” family

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 “Old Yellow Enzyme” (OYE) was discovered from brewer’s bottom yeast as the first flavin-binding protein in 1932 by O. Warburg and W. Christian. However, its physiological and biochemical functions have been unrevealed to date. Recently, an OYE-homologous protein in *Arabidopsis thaliana* was identified as 12-oxo-phytyldienoate reductase (OPR) which catalyzes a reductive reaction in jasmonate synthetic pathway in vascular plants. OYE homologous proteins also distribute in eubacteria and protozoa and some of these proteins have a catalytic activity for reduction of alpha-beta unsaturated compounds: e. g., an OYE-homologous

protein in *Trypanosoma cruzi* plays an important role in a prostaglandin synthesis.

We found a novel OYE-homologous protein in the flagellar fraction from phototactic zoospores of a marine macrophytic brown alga, *Scytosiphon lomentaria* (Fujita et al. (2005) European Journal of Phycology, *in press*). The posterior flagellum of the zoospore exhibits green autofluorescence which seems to be related with a photoreceptor for phototaxis. We isolated flagella from zoospores discharged from field-collected thalli of *S. lomentaria* and partially purified a flavoprotein by liquid chromatography. Spectrofluorometric and chromatographic analyses revealed that this flavoprotein noncovalently bound FMN as a chromophore and showed a molecular mass of 41 kDa. Based on internal amino acid sequences determined by protein sequencing after in-gel digestion, a full-length cDNA of the 41-kDa flavoprotein was cloned and sequenced. Deduced amino acid sequence of the 41-kDa flavoprotein showed a significant similarity with OYE and its homologous proteins. We will discuss molecular structure, physiological function and phylogenetical relationship of the flavoprotein in comparison with other members of OYE family.

PII85

Peridinin triplet state dynamics in Peridinin–Chlorophyll-a–Protein (PCP)

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 With the investigation of the peridinin triplet state (³Per) dynamics of the light-harvesting (LH) complex PCP, we aim to better understand the photo-protection mechanism of LH complexes by carotenoids. In this time-resolved IR-spectroscopic study, we distinguish several Per conformers and correlate them to the PCP X-ray structure. We identify two components with lifetimes of ~10 and ~40 μ s, both showing Per and Chl-a modes. This indicates the involvement of Chl-a in the ³Per dynamics. The amplitude and some spectral features of only the slower component are wavelength dependent. Hence, ³Per triplets are formed via two different pathways: The faster component represents the lifetime of a typical carotenoid triplet formed by quenching Chl-a triplets, the 40 μ s component is the result of a special mechanism owing to Per’s particular chemical structure and its tuning-to-function by the protein environment and Chl-a, providing in an efficient photo-protection mechanism.

PII86

Ultrafast events in the Photoactive Yellow Protein chromophore: protein vs solution environment

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 The Photoactive Yellow Protein (PYP) is the photoreceptor protein responsible for the photomovement of the bacterium *Halorhodospira Halophila*. It contains a 4-hydroxycinnamoyl chromophore covalently linked to the side chain of residue Cys69 by a thioester bond. Upon irradiation with blue light, PYP undergoes a photocycle involving both chromophore and protein structural relaxations and characterized by several spectroscopic intermediates formed on timescales spanning from several hundred femtoseconds to seconds. *Trans-cis* photoisomerization of the chromophore was clearly identified as the first overall molecular process of the photocycle, the formation of a relaxed *cis* isomer (I₁) arising in a few nanoseconds, but the internal and external

coordinates involved in the photoisomerization reaction path on the femto-picosecond timescale are still debated (for a review, see K. J. Hellingwerf *et al.*, *J. Phys. Chem. A* 2003, 107, 1082).

We will report a comparative study of the isomerization reaction in native PYP and in various chromophore analogues in solution (P. Changenet-Barret *et al.*, *New J. Chem.* 2005, 29, 527). The reaction dynamics was followed by ultrafast transient absorption spectroscopy. The results will be discussed in terms of (i) the intrinsic photophysics of the chromophore and (ii) the geometrical constraints imposed by the native environment.

PII87

Structure, energetics and spectra of the most stable isomers of some $C_{60}X_{12}$ and $C_{60}X_6Y_6$ heterofullerenes

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In this paper we report the structure and the energies of the more stable isomers of fullerene derivatives $C_{60}N_{12}$, $C_{60}B_{12}$ and $C_{60}B_6N_6$. The calculations have been performed at the B3LYP/6-31G* level. For each isomer the vibrational IR and Raman spectra have been computed to provide a tool to identify the forms that will be synthesized. The HOMO-LUMO energy gap, which is a measure of the ability of a compound to act as a semiconductor, was evaluated together with charge distribution. Some isomers of these heterofullerenes are expected to have more interesting properties than C_{60} . For some of the cages we have evaluated the electronic excitation energies and modelled the absorption spectra. In all the systems the lowest bands appear to move to lower energies a results of the symmetry lowering with respect to C_{60} . Furthermore, as expected, the lowest S_0 - S_1 energy gap is found to be smaller for the less stable isomers.

PII88

Regioselective self-assembly of zinc 3-hydroxymethyl-13-formyl-chlorin and the corresponding 3,13-inverted pigment

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Photosynthetic green bacteria have unique light-harvesting antennas, so-called chlorosomes, in which bacteriochlorophyll(BChl)s-*c/d/e* self-assembled by specific interaction among 3¹-OH, central magnesium and 13-C=O moieties. Zinc chlorins possessing 3-C=O and 13¹-OH (inverted-type pigment) easily formed large aggregates as well as zinc 3¹-hydroxy-13¹-oxo-chlorins (normal-type), indicating that the linear situation of the three substituents on its Qy-axis is required for chlorosomal self-assembly (Yagai, S.; Miyatake, T.; Tamiaki, H. *J. Org. Chem.* 2002, 67, 49-58). Here we report synthesis and chlorosomal self-assembly of zinc 3-hydroxymethyl-13-formyl- and 3-formyl-13-hydroxymethyl-chlorins **1** and **2**.

The normal chlorosomal BChl model **1** and the inverted-type **2** were synthesized by chemical modification of rhodochlorin XV dimethyl ester. Monomeric absorption spectra of **1/2** in THF showed a small regioisomeric difference; the Qy maxima of **1** situated at 643 nm and that of **2** red-shifted to 651 nm. Moreover, their oligomeric properties in aqueous media showed a remarkably large difference; normal-type **1** easily formed large self-aggregates possessing red-shifted Qy maximum at 722 nm whereas inverted-type **2** formed dimer-like small aggregates as indicated by less red-shifted Qy maximum at 686 nm. The regioselective control on self-aggregation will be discussed by electronic, circular dichroism and FT-IR absorption and fluorescence emission spectroscopies. Present models **1** and **2** lacking an exo-five-membered ring characteristic of naturally occurring chlorophylls, should be more useful to observe essential difference on the self-aggregation of normal- and inverted-type pigments than the previous.

PII89

Disturbance of chlorophyll formation at the level of 5-aminolevulinic acid and magnesium-porphyrins biosynthesis in isogenic lines of spring wheat (*Triticum aestivum* L.) marked by genes *cn-A1* and *cn-D1*

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The *cn-A1* and *cn-D1* markers in almost isogenic lines of wheat (*Triticum aestivum* L.) ANK-32A (*cn-A1*) and ANK-32B (*cn-D1*) result in change of an ratio between the photoactive and photoinactive forms of protochlorophyllide in etiolated leaves. The rate of protochlorophyllide resynthesis in short illuminated etiolated leaves of ANK-32A and ANK-32B as well as the rate of 5-aminolevulinic acid (ALA) and chlorophyll biosynthesis in such greening seedlings are reduced. In conditions of not limited supply of the leaves with exogenous ALA the marked lines accumulate the smaller amount of protochlorophyllide, sum of Mg-protoporphyrin IX and its monomethyl ether on a background of higher content of their precursor, protoporphyrin IX. It was concluded that chlorophyll deficiency of ANK-32A and ANK-32B lines of wheat is a result from reduces capacity to ALA synthesis and decrease in enzymatic activity on a step of incorporation of Mg ions in the protoporphyrin IX ring.

PII90

Self-assembly of amphiphilic zinc chlorins possessing a hydrophilic oligooxyethylene group

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Green photosynthetic bacterium has a unique light-harvesting antenna called chlorosome, in which a number of bacteriochlorophyll(BChl)s-*c, d, e* molecules self-assemble to form J-aggregate with a red-shifted Qy absorption band. In order to mimic the chlorosome-type antenna system, zinc chlorins possessing 3¹-hydroxy and 13-keto groups were prepared as model compounds for BChls-*c, d, e*. The synthetic pigments self-aggregated to form chlorosome-like aggregates, which worked as an artificial light-harvesting antenna systemⁱ. Here we report aqueous assemblies of amphiphilic zinc chlorins possessing a hydrophilic oligooxyethylene chainⁱⁱ.

A hydrophilic oligooxyethylene group, $-(CH_2CH_2O)_nH$, was introduced into a zinc chlorin moiety as an esterified chain at the 17-position. The amphiphilic zinc chlorin **1** preferentially gave small aggregates such as dimer in an aqueous medium. When the terminal hydroxy group of the oligooxyethylene chain in **1** was end-chapped with an acetyl group to form zinc chlorin **2** with $-(CH_2CH_2O)_nAc$, the amphiphilic pigment formed large aggregate with a Qy band at around 730 nm. Thus, the aqueous aggregate of amphiphilic zinc chlorin **2** afforded a good model for chlorosomal aggregates. In addition, an oligooxyethylene with monohexadecyl ether, $-(CH_2CH_2O)_n(CH_2)_{15}CH_3$, was introduced into a zinc chlorin moiety as an esterified chain to give **3**. The amphiphilic pigment formed chlorosome-type aggregates in an aqueous medium, and the intensities of their CD signals and fluorescence emission were larger than those of aggregated **2**. Thus, the aqueous aggregates of **3** might have well ordered suprastructures.

ⁱ T. Miyatake, H. Tamiaki, A. R. Holzwarth & K. Schaffner, *Helv. Chim. Acta*, 82, 797 (1999).

ⁱⁱ T. Miyatake, H. Tamiaki, H. Shinoda, M. Fujiwara & T. Matsushita, *Tetrahedron*, 58, 9989 (2002).

PII91

Supramolecular gels prepared with self-assembly of amphiphilic zinc chlorins

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In a chlorosomes of green photosynthetic bacterium, a number of bacteriochlorophyll (BChl)s-c, d, e molecules self-aggregate to form rod-like oligomers. Synthetic zinc chlorins afforded chlorosome-type self-aggregates in a non-polar organic solvent such as hexane. The artificial aggregate provides a good structural and functional model for chlorosome¹.

A hydrophilic oligooxyethylene group was introduced into a zinc chlorin moiety as an esterified alkyl chain at the 17-position, and the terminal hydroxyl group of oxyethylene chain was end-capped with acetyl group to give amphiphilic zinc chlorin. The amphiphilic pigment self-assembled to form a micelle-like aggregate in a diluted aqueous medium ([zinc chlorin] = 10 µM). In contrast, the zinc chlorin aggregates gave supramolecular gels in a concentrated condition ([zinc chlorin] > 5 mM). A methanol solution of amphiphilic zinc chlorin was diluted with water. Then, the obtained aggregate solution was heated at 50 °C to form gel. Absorption spectrum of the zinc chlorin gel showed a red-shifted Qy band at around 710 – 740 nm, suggesting that the zinc chlorin moiety self-assembled to form J-aggregate as well as natural BChls-c, d, e do in a chlorosome. In addition, zinc chlorin dyads in which two zinc chlorin moieties were connected with an oxyethylene linkage were prepared. The amphiphilic dyads also formed supramolecular gel with a red-shifted Qy band.

¹ T. Miyatake, H. Tamiaki, A. R. Holzwarth & K. Schaffner, *Photochem. Photobiol.*, 69, 448 (1999).

PII92

Streptomycin effects on the activity of chlorophyll biosynthesis in barley seedlings

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Exposure of barley seeds to streptomycin (Sm) prior to germination induces the development of *albino* seedlings with undifferentiated ribosome-deficient plastids. These plants are useful tool for investigation of the influence of developmental and metabolic states of plastids on the expression of nuclear genes encoding chloroplast proteins. The effects of Sm on the activity of chlorophyll (Chl) biosynthesis in barley seedlings were investigated. Sm-treated leaves contained 0.5% of control Chl content. The capacity of *albino* seedlings to synthesize the first Chl precursor, 5-aminolevulinic acid (ALA) in the light amounted to 3.5% of that in green leaves. The darkening of green control leaves resulted in decrease of ALA synthesis rate by 25 times. *Albino* plants accumulated similar amounts of ALA in the light and in the dark, indicating light-independent synthesis of ALA in Chl-deficient leaves. The lower activity of ALA dehydratase (ALAD) was also determined in white seedlings (66% of the control activity). Feeding barley leaves with exogenous ALA in the dark caused accumulation of Chl precursors, the total amount of which was approximately 10-times lower in Sm-treated leaves. A part of protoporphyrin IX in the total pool of porphyrins synthesized from ALA was 80% and 40% in etiolated and light-grown Sm-treated seedlings, respectively, while in etiolated and green control leaves it amounted 20-22%. These results are indicative for decreased activity of Mg-chelatase and its activation upon illumination in leaves of *albino* phenotype. Thus, Sm almost completely inhibited ALA-synthesizing capacity as well as reduced the activity of the subsequent enzymes of Chl pathway, ALAD and Mg-chelatase.

PII93

Regulation of 5-aminolevulinic acid synthesis in roots of barley seedlings

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The synthesis of 5-aminolevulinic acid (ALA) is the rate-limiting step for the formation of all plant tetrapyrroles, including chlorophyll and heme. ALA synthesis is tightly regulated through the co-ordinated action of a wide range of signals. These include light, cytokinins, the circadian clock, temperature, photooxidative stress and feedback control. Compared with the regulation of ALA synthesis in photosynthetic plant organs, the role of various regulatory factors on ALA synthesis in non-photosynthetic plant tissues is much less investigated. Effects of light, kinetin and metal chelator 2,2'-dipyridyl (DP) on ALA synthesis in roots of barley seedlings were examined. Light exposure of cut roots of etiolated barley seedlings did not affect their ALA-synthesizing capacity. Kinetin did not also influence the rate of ALA synthesis in barley roots. Since the roots are the organs of preferential heme production, these results indicate that light and cytokinins are not essential for regulation of ALA formation assigned for heme biosynthetic pathway. Incubation of cut roots of green barley seedlings with DP in the dark resulted in increase or decrease of ALA synthesis rate compared with control. The effect depended on the DP concentration. Application of 0.001 mM or 0.01 mM DP enhanced the ALA-synthesizing capacity by 66 % and 30 %, respectively, whereas increase DP concentration till 1–3 mM resulted in 2-times inhibition of ALA synthesis compared with control. The level of glutamate 1-semialdehyde aminotransferase was enhanced in DP-treated roots upon all used concentration of chelator. DP decreased the amounts of non-covalently bound heme by 25 % in all variants in comparison with control. Thus the reduction in heme content by DP treatment led to stimulated ALA synthesis in roots of green barley seedlings. These results present the argument in favor of heme as a significant feedback inhibitor of ALA synthesis in roots.

PII94

Seasonal dynamics of xanthophyll cycle pigments in lichen *Xanthoria parietina*

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Lichen photobionts possess various photoprotective mechanisms. One of these is xanthophyll cycle (Demmig-Adams & Adams 1992). Protective roles of xanthophylls include heat dissipation of excess energy (reviewed by e.g. Gruszecki 1995) and scavenging of reactive oxygen species (Havaux & Niyogi 1999). Zeaxanthin (Z) content and total xanthophyll pool content (violaxanthin, antheraxanthin, zeaxanthin - VAZ) rise under such conditions when the amount of absorbed light energy exceeds the energy that can be utilized in metabolic processes (Demmig-Adams & Adams 1996).

To evaluate seasonal dynamics, we collected thalli of *X. parietina* on 10 collection dates during the period from March 2003 to February 2004 and determined xanthophyll cycle pigments and chlorophyll (Chl) *a* and *b*, by HPLC. Additionally, the functionality of photosynthetic apparatus was monitored by several Chl fluorescence parameters.

The thalli collected in winter contained low amounts of VAZ. During spring, VAZ pool increased, with a peak found in early May. In summer and early autumn, the VAZ content was constant, followed by a decrease from late autumn until winter solstice. The de-epoxidation state of xanthophyll cycle pigments (DEPS, calculated as Z/(V+A+Z)) and maximal quantum yield of photosystem II (F_v/F_M) showed similar dynamics during majority

of the year. The Z content closely followed the DEPS. Total Chl content increased from the end of March to a maximum found in winter. The Chl a/b ratio rose from winter to summer. Obtained data show that the *X. parietina* photobiont acclimates to seasonal changes in the site microclimate of well-lit habitats.

References: Demmig-Adams B & Adams WW III (1992) *Annu Rev Plant Physiol Plant Mol Biol* 43, 599-626
 Demmig-Adams B & Adams WW (1996) *Trends in Plant Sci* 1, 21-26
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PII95

Photosynthetic characterization of the seagrass *Cymodocea nodosa* along depth and within leaf gradients

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Seagrasses are the only group of vascular plants that has colonized successfully marine environments and can survive in a wide range of emersion conditions. Depth-related changes in photosynthetic physiology were studied in populations of the seagrass *Cymodocea nodosa* from Cadiz Bay Natural Park (Southern Spain), located in a depth gradient from the intertidal to the subtidal. In addition, within leaf variability was also assessed at each depth. The PE (photosynthesis vs irradiance) curves were carried out through simultaneous measurements of oxygen evolution (Hansatech oxygen chamber) and variable fluorescence (PAM fluorometer). The net maximum photosynthesis rate (Pmax) was unaffected by depth. In contrast, Pmax on area basis was lower in the leaf apex than in the base, irrespective of depth location of the plants. Likewise, photosynthetic efficiency (α) was unaffected by light gradients but depends on the position within the plant, being lower in apical parts. At difference with oxygen measurements, the maximum quantum yield (Fv/Fm) for fluorescence increased along the depth gradient, and was also affected by the position within the leaf, being lower in the apex, specially in intertidal plants. The effective quantum yield ($\Delta F'/Fm$) dropped along photon irradiances. This decline was more pronounced in leaf tips than in the base in the three stations analyzed. These photosynthetic responses are linked to morphological and population characteristics. In this way, leaf length, width and thickness increased along depth. There were also changes in thickness along the leaves. All the morphological and photosynthetic characteristics result in lower biomass but higher shoot densities in intertidal than in subtidal populations. Additional results on pigment content and growth rate are also reported.

PII96

Activation of photosynthetic electron transport and differential expression of proteins in rice (*Oryza sativa* L.) leaves by photocatalyst (TiO₂)

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Photocatalyst is a hopeful technology based on the interaction between light and solid semiconductor particles and is able to produce highly oxidative species that not only destroy bacteria, but also destroys a large variety of chemical contaminants in water. Among the photoactive semiconductors are namely TiO₂, ZnO, Fe₂O₃, WO₃ and CdSe. TiO₂ is most widely used in different media as photocatalyst, because of its lack of toxicity and its stability. In this study, effect of photocatalyst (TiO₂) were investigated to obtain information of physiological change in rice plant. Chlorophyll fluorescence index and relative electron transport rate (ETR) of rice

leaves were activated by TiO₂. Relative electron transport rate were TiO₂ (45.1%) > control (32.4%) > diuron (electron transport inhibitor (15.3%)), open field > glasshouse > 75% shade in glasshouse also. Intensity of day accumulated ultra violet B were open field : 13.6kJ, glasshouse : 0.6, 75% shade in glasshouse: 0 kJ at July in Korea. Proteins extracted from leaves were separated by two-dimensional polyacrylamide gel electrophoresis. Protein spots were identified by matrix-assisted laser desorption ionization-time of flight (MALDI-ToF) mass spectrometry. We used a protein database from NCBI to identify proteins. TiO₂ changed the intensity of seventy proteins and thirty proteins out of them were identified: sixteen proteins (NAD(H)-plastoquinone oxidoreductase subunit J, putative thioredoxin peroxidase, putative cell death associated protein, putative glucosyltransferase, putative methionine S-methyltransferase, putative cinnamyl alcohol dehydrogenase, etc) were up-regulated and fourteen proteins (lipoxygenase, putative elicitor-inducible cytochrome p 450, sucrose-UDP glucosyltransferase 2, putative transketolase, putative ethylene-inducible protein, etc) were down-regulated.

Keywords: photocatalyst, TiO₂, relative electron transport rate, rice.

PII97

Isolation and characterization of a novel photomorphogenic and circadian clock mutant in *Arabidopsis*

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Many physiological and biochemical processes in plants exhibit endogenous rhythms with a period of about 24 hours. These rhythms are generated and maintained by an internal timing mechanism, the circadian clock, which enables plants to anticipate the forthcoming rhythmic changes in the environment and to temporally coordinate their internal processes. In order to learn more about the molecular structure and function of the circadian clock in *Arabidopsis*, a luciferase imaging-based mutant screen has been carried out and several mutants showing aberrant rhythmic expression of the *CAB2* gene (coding for the CHLOROPHYLL A/B-BINDING protein 2) have been identified. As a result of this screen we have identified a mutation, *B6*, which shortens the period length of many clock-controlled processes - including the expression of several genes and the rhythm of leaf movement - under various free-running conditions. The *B6* mutant also shows altered photomorphogenic responses: (i) the hypocotyls of the mutant seedlings are shorter, when they are grown in red or blue light; (ii) the cotyledons of *B6* are smaller under all light qualities and intensities examined; (iii) the cotyledon angle is smaller in *B6*; (iv) the mutant plants accumulate more anthocyanin than wild-type plants.

The mutation is mapped to a region of chromosome 5, where already known clock genes are located. However, comparative sequencing of those clock genes revealed that they are unchanged in the mutant, qualifying *B6* as a novel component of the *Arabidopsis* circadian clock and the light signaling mechanism.

PII98

New light signalling component affecting circadian clock in *Arabidopsis thaliana*

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It is a well-known phenomenon that some biological processes exhibit periodic changes on a daily time-scale even in the absence of external periodic stimuli. This can be attributable to the

functioning of an internal molecular pacemaker called 'circadian clock'. Circadian clocks are thought to provide higher fitness to an organism by synchronising internal processes to the periodic changes in the environment.

To identify new circadian clock elements in the model organism *Arabidopsis thaliana*, we have initiated a genetic screen. We have searched for circadian clock mutants exhibiting altered circadian expression pattern of the *CAB2* (coding for the CHLOROPHYLL A/B-BINDING protein 2):luciferase marker gene. As a result of this screen we have identified a mutation, *Rs2*, which lengthens the period of many clock-controlled processes – including the expression of several genes and the rhythm of leaf movement – under various environmental conditions. The *Rs2* mutant doesn't show altered photomorphogenic responses regarding to hypocotyl elongation, and cotyledon opening, but the expansion of cotyledons is enhanced in the mutant under red light. Moreover, acute induction of *CAB2* expression by red light is increased in *Rs2* about four-fold, suggesting an important role of this molecule in red light signalling.

The mutation is mapped to the bottom of chromosome 5, where already known clock genes are located. However, comparative sequencing of these genes revealed that they are unchanged in the mutant, qualifying *Rs2* as a novel component of the *Arabidopsis* circadian clock.

PII99

Analysis of the maize leaf proteome after various UV-B treatments of lines differing in UV-B sensitivity

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We systematically surveyed responses of maize leaves to UV-B radiation using DIGE 2D gels followed by identification of selected proteins by MS/MS and Western blot analysis. Given its heightened sensitivity to UV-B the *b, pl* W23 flavonoid-deficient line was compared to two maize landraces from high altitudes (Cacahuacintle, and Confite Puneño) that have improved UV-B tolerance. Protein patterns in adult maize leaves (*Zea mays*) were documented after growth for 21 days in sunlight depleted of UV-B or growth in sunlight including an 8h UV-B supplementation during one day in the field. We found that there is a very high correlation between mRNA accumulation assessed by microarray hybridization and qRT-PCR and protein expression after UV-B irradiation in leaves of W23. Multiple isoforms were confirmed for some proteins; at least one protein, pyruvate phosphate dikinase (PPDK), is regulated post-translationally by reversible phosphorylation during UV-B exposure. Proteins differentially regulated by UV-B in W23 with higher levels under similar UV-B conditions in high altitude plants were also identified. These can be genetically fixed traits conferring UV-B tolerance and are specific adaptations to living at high altitude.

PII100

Expression of genes for early light inducible proteins under oxidative stress in barley

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During photosynthesis plants absorb light; however, excess light leads to inhibition of photosynthesis and irreversible photooxidative destruction of plastids. The production of light stress proteins (ELIP-Early Light-Inducible Protein) is one of the defense mechanisms evolved by plants to protect themselves against photodestruction. In higher plants, the proteins of the ELIP family are nuclear-encoded, synthesized on the cytoplasmic ribosomes, and imported into the chloroplasts as polypeptide precursors. Plastid-to-nucleus signaling was shown for

photosynthetic genes. Tetrapyrroles and proteins were suggested to be involved in this process. The redox state of plastids is also important. The mechanisms controlling expression of light stress genes are poorly understood. The role of chloroplasts in the transcriptional regulation of ELIP genes under norflurazon-induced oxidative stress was studied. In plants grown in the presence of norflurazon, the synthesis of carotenoids is inhibited. In the absence of carotenoids, oxidative stress develops leading to photodestruction of chloroplasts. The finding that at photodestruction of chloroplasts, transcription of the ELIP genes is decreased indicates that their expression is chloroplast-regulated. However this effect is less pronounced for the ELIP genes than for the photosynthetic genes *Lhcb* and *RbcS*. The experiments with dipyrindyl, which inhibits the Mg-Protoporphyrin IX monomethylester cyclase, showed that regulation of the ELIP gene transcription is mediated by tetrapyrroles, which cause transcription inhibition by 30-50%. In summary, our results demonstrate that chloroplasts are involved in regulation of light stress protein genes. Tetrapyrroles play an important role in this process.

This work was supported by the Russian Foundation for Basic Research (project no. 03-04-49051).

PII101

Photooxidative stress in barley leaves treated with Rosa Bengal

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The xanthene dyes are a commonly used group of photodynamic sensitizers acting through a predominantly type II mechanism. Rose bengal (RB), tetra-iodo-tetrachlorofluorescein, is the most efficient molecular singlet oxygen producer. The illumination of green barley leaves treated with RB in the dark resulted in development of photooxidative stress. After 1 h of illumination the level of reactive oxygen species (ROS) slightly increased in leaves incubated with RB. The contents of TBA-products, chlorophyll (Chl) and carotenoids (Car) were did not practically differ from control values. However parameters of photosystem 2 activity such as electron transport, photochemical quenching of Chl fluorescence and quantum yield of photochemistry of photosystem were reduced. Longer illumination of RB-treated leaves (for 8 h) led to increase of ROS level by 1.4 times and TBA-products content by 1.7 times compared with the control accordingly. RB-treated leaves contained 70 and 80% of control Chl and Car content respectively. The further illumination (for 24 h) resulted in two times increase of ROS level compared with the control. The ROS accumulation was accompanied by photodynamic destruction of Chl and Car by 55 and 40% respectively, and by increase of TBA-products by 200% in comparison to the control. Low temperature fluorescence spectra (-196°C) showed that illumination for 24 h led to considerable decrease of the energy transfer in photosystems and resulted in destruction of the both photosystems in leaves treated with RB.

IL: Invited Lecture; OC: Oral Communication; PL: Plenary Lecture; PI/II: Poster session I/II

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